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(54) **Title:** HUMAN ANTI-ADIPOCYTE MONOCLONAL ANTIBODIES AND THEIR USE

(57) **Abstract:** A panel or library of antibodies that bind adipocyte antigens, methods of obtaining isolated antibodies and antibody variable domains and mixtures thereof, uses in identifying antigen molecules, in assays, diagnostically and therapeutically.

HUMAN ANTI-ADIPOCYTE MONOCLONAL ANTIBODIES AND THEIR USE

The present invention relates to antibodies directed to adipocytes and particular antigens on adipocyte surfaces. It further relates to libraries and panels of antibodies which may be screened for identification of antibodies useful in particular contexts, which may be used in a variety of contexts, including identifying adipocyte antigens.

Obesity is a disease characterised by a pathological increase in adipose cell mass. As a disease it is becoming more prevalent, with over 50% of adults in the UK already overweight and 1 in 5 of those now clinically obese. Consequently, over recent years there has been a marked increase in adipocyte research, the cell type responsible for storage of excess lipid. For many years the adipocyte was believed to be a relatively dormant cell type, acquiring or losing lipid stores in response to regulatory signals from other cells. However, recent research has demonstrated that adipose tissue is actively secreting metabolic regulators and that these play a key role in energy homeostasis and food intake.

The most well known adipocyte signalling molecule identified to date is leptin. Leptin, a 16KDa protein encoded by the ob gene, is secreted by adipocytes in concentrations directly proportional to body fat mass (Zhang et al., (1994) Nature 372: 425-432; Rosenbaum et al., (1996) J. Clin. Endocrinol. Metab. 81: 3424-3427). Although the biological role of leptin has not been fully deduced, it has been shown in mice to bind a receptor in the hypothalamus and induce satiety (Tartaglia et al., (1995) Cell 83: 1263-1271; Chen et al., (1996) Cell 84: 491-495; Lee et al., (1996) Nature 379: 632-635). Systemic or intracerebroventricular administration of leptin decreases food intake and results in reduced body fat

(Schwartz et al., (1996) Diabetes 45: 531-535; Halaas et al., (1995) Science 269: 543-546; Pelleymounter et al., (1995) Science 269: 540-543; Campfield et al., (1995) Science 269: 546-549). Moreover, in ob-ob mice, which are very obese, the ob gene is mutated so that no leptin is produced; when administered leptin, the mice stop eating and rapidly lose weight (Pelleymounter et al., (1995) Science 269: 540-543; Halaas et al., (1995) Science 269: 543-546). One of the ways in which leptin acts is by down-regulating expression of the appetite-stimulating peptide NPY (neuropeptide-Y) (Stephens et al., (1995) Nature 377: 530-532; Schwartz et al., (1996) Diabetes 45: 531-535). High concentrations of NPY promote eating, and intracerebroventricular infusions of NPY can cause obesity in normal rats (Stanley et al., (1986) Peptides 7: 1189-119). In ob-ob mice, the beneficial effects of administering leptin are accompanied by a marked decrease in hypothalamic NPY concentrations (Stephens et al., (1995) Nature 377: 530-532). However, transgenic mice lacking the NPY gene still respond to the effects of leptin suggesting that it acts through other mechanisms independent of NPY (Erickson (1996) Nature 381: 415-421).

Although the discovery of leptin and its effect on obese rodents has led to renewed interest in adipocyte biochemistry, the effect of leptin in human obesity is less well understood. In fact, there appears to be little correlation between obese rodents and obese humans, for example administration of leptin to obese patients has little effect on body weight and also there have only been isolated accounts of ob-ob human equivalents (Montague et al., (1997) Nature 387: 903-908). Thus there remains much to be discovered regarding adipocyte biology and the mechanisms of obesity in humans.

One area of adipocyte biology that is relatively

uncharacterised is the adipocyte cell surface. Indeed, a more complete understanding of the proteins and receptors expressed on the adipocyte cell surface may provide a major insight into the causes of obesity. A few receptors have already been identified on adipocytes, for example the insulin receptor, the leptin receptor and also several fatty acid transporters (Reed et al., (1977) PNAS 74(11): 4876-4880; Lefebvre et al., (1998) Diabetes 47: 98-103; Tartaglia et al., (1995) Cell 83: 1263-1271; Rosenbaum et al., (1997) New Eng. J. Med. 337: 396-407; Hui and Bernlohr (1997) Frontiers in Bioscience 2: 222-231). In addition, up to 20% of the adipocyte cell surface is comprised of surface invaginations of the plasma membrane known as caveolae (Lisanti et al., (1994) Trends Cell Biol. 4: 231-235). These structures are unique in that they are rich in signalling molecules and their cognate receptors, including G proteins, Src-like kinases, protein kinase C and Ras-related GTPases (Sargiacomo et al., (1993) J. Cell Biol. 122: 789-807; Lisanti et al., (1994) J. Cell Biol. 126: 111-126; Chun et al., (1994) PNAS 91: 11728-11732; Chang et al., (1994) J. Cell Biol. 126: 127-138; Shenoy-Scaria et al., (1994) J. Cell Biol. 126: 353-363; Robbins et al., (1995) Mol. Cell Biol. 15: 3507-3515; Schnitzer et al., (1995) Science 269: 1435-1439). When one also remembers that adipocytes secrete many soluble factors, for example leptin, TNF-α and adipsin (Zhang et al., (1994) Nature 372: 425-432; Hotamisligil et al., (1993) Science 259: 87-91; Cook et al., (1985) PNAS 82: 6480-6484), it is clear that although adipocytes are a metabolically active cell type, relatively little is known about their cell surface signalling mechanisms.

Adipocytes are a unique cell type in that they can store large quantities of lipid and it is likely that this phenotype is reflected in the enzyme and receptor composition of the plasma membrane. One approach that has been used to

further our knowledge of the adipocyte cell surface is the production of antibodies to plasma membrane antigens. Antibodies have proven to be highly useful research tools over the years due to the variety of techniques in which they can be applied; for example Western blotting, immunocytochemistry, ELISA, immunopreciptation, affinity chromatography to name but a few. This versatility has led to the use of antibodies in many characterisation studies, for example in the identification of disease and cell differentiation/development markers. Thus, in effect, a panel of antibodies directed against adipocyte plasma membranes would be a toolkit with which to study adipocyte biology.

Anti-adipocyte antibodies have been generated by immunising animals with adipocyte plasma membranes isolated from various species (rats, cattle and sheep) (Pillion et al., (1979) J. Biol Chem. 254: 3211-3220; Thompson et al., (1979) In Vitro 15: 441-445; Lee et al., (1986) J. Dev. Physiol. 8: 207-226; Cryer et al., (1984) J. Dev. Physiol. 6: 159-176; Nassar AH, Thesis, Corvallis, OR: Oregon State University, 1989; Flint et al., (1986) Int. J. Obesity 10: 69-77). Although polyclonal sera have been used to identify adipocyte membrane proteins, this approach is generally complicated by a number of factors, such as differing antibody affinities and isotypes, variability in serum samples and production of antibodies to intracellular epitopes. An alternative approach is to use monoclonal antibodies rather than polyclonal antisera to study adipocyte membrane proteins (Killefer and Hu (1990) PSEBM 194: 172-176; Wright and Hausman (1995) Obesity Research 3: 265-272; De clercq et al., (1997) J. Anim. Sci. 75: 1791-1797).

To isolate antibodies from a phage library (McCafferty et al., (1990) Nature 348: 552-554; Johnson and Chiswell (1993) Current Opinion in Structural Biology 3: 564-571; Winter et

al., (1994) Ann. Rev. Immunol. 12: 433-455), typical selection techniques involve immobilising a purified antigen on a solid support and then panning with the antibody library (Vaughan et al., (1996) Nature Biotechnology 14: 309-314). Whilst this works well for purified antigens, selection procedures for more complex antigens are less well developed. Phage antibodies have been isolated to red blood cells, leukocytes, epithelial cells and tumor cells (Marks et al., (1993) Biotechnology 11: 1145-1149; De Kruif et al., (1995) PNAS 92: 3938-3942; Palmer et al., (1997) Immunology 91: 473-478; Watters et al., (1997) Immunotechnology 3: 21-29), but only limited selections have been described with regard to selections on antigenically complex targets such as the cell surface.

The present invention has arisen by provision of a panel of anti-adipocyte monoclonal antibodies binding to many different antigens on the adipocyte cell surface. The antibodies have been characterised by immunocytochemistry on a panel of normal human tissues to establish tissue and cell type specificity and allow for antigens of interest to be characterised and subsequently identified. These anti-adipocyte antibodies may also be used in the treatment of obesity and obesity related diseases. One advantage of this approach is that antibodies specific for different fat depots may be produced: for example, intra-abdominal fat is associated with many of the complications of obesity (NIDDM, hypertension, heart disease and colon cancer); alternatively recurrent lipoma (benign fatty tumours) may be controlled using specific antibodies; other cardiovascular conditions associated with obesity such as atherosclerosis may be targeted; and more unusual conditions such as thyroid eye disease, where patients suffer from an increase in adipose mass around the eye-ball and is currently only treatable by surgery. Antibodies may be used to deliver drugs or

pro-drugs directly to the fat mass of an obese patient or alternatively an antibody with an appropriate specificity may be used as a therapeutic itself. For example, antibodies binding specifically to adipocytes may be used to activate the immune system to destroy the cells by complement mediated lysis (Marks et al., (1993) Biotechnology 11: 1145-1149; Wright and Hausman (1995) Obesity Research 3: 265-272; De clercq et al., (1997) J. Anim. Sci. 75: 1791-1797). Using antibodies that can target adipocytes offers an alternative means of treatment for obese patients other than undergoing surgery to remove excess fat.

In one aspect, the present invention provides a library or panel of at least or about 10 different specific binding members, the library or panel comprising specific binding members each able to bind whole adipocytes and each comprising an antibody VH variable domain, wherein each antibody VH variable domain comprises a VH CDR shown in Table 4 and optionally has an amino acid sequence selected from the group consisting of those with a SEQ ID NO. listed in Table 5.

Such a library may include at least or about 20 different antibody VH CDR's or variable domains, or at least 50, 60, 70, 80, 90 or 100 different antibody VH CDR's or variable domains.

In one embodiment, the library contains all 108 different VH domains of which the amino acid SEQ ID NO.'s are listed in Table 5. In another embodiment, the library contains all 108 different VH CDR3's shown in Table 4.

Generally, each VH domain is paired with a VL domain. The VL domain may be any selected from those disclosed herein, e.g. with a SEQ ID NO. listed in Table 5. Preferred VL domains

for inclusion in a library or specific binding member according to the present invention include those with SEQ ID NO.'S 4, 8, 26, 84, 88 and 116, especially SEQ ID NO. 26 and SEQ ID NO. 84.

In a further aspect, the present invention provides a method of obtaining one or more specific binding members able to bind an adipocyte antigen, the method including bringing into contact a library of specific binding members according to the invention and said adipocyte antigen, and selecting one or more specific binding members of the library able to bind said adipocyte antigen.

The library may be displayed on the surface of bacteriophage particles, each particle containing nucleic acid encoding the antibody VH variable domain displayed on its surface, and optionally also a displayed VL domain if present.

Following selection of specific binding members able to bind the antigen and displayed on bacteriophage particles, nucleic acid may be taken from a bacteriophage particle displaying a said selected specific binding member. Such nucleic acid may be used in subsequent production of a specific binding member or an antibody VH variable domain (optionally an antibody VL variable domain) by expression from nucleic acid with the sequence of nucleic acid taken from a bacteriophage particle displaying a said selected specific binding member.

An antibody VH variable domain with the amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in isolated form, as may a specific binding member comprising such a VH domain.

A plurality of antibody VH variable domains each with an amino acid sequence of an antibody VH variable domain of a

said selected specific binding member may be provided in isolated form, as may a plurality of specific binding members comprising such VH domains.

A mixture of said plurality of antibody VH variable domains may be provided in isolated form.

An antibody VH variable domain with an amino acid sequence of an antibody VH variable domain of a said selected specific binding member, a plurality of said antibody VH variable domains, or a mixture of a plurality of said antibody VH variable domains in isolated form may be formulated into a composition including at least one additional component, for instance a composition including a pharmaceutically acceptable excipient. The same applies to specific binding members comprising a VH domain and optionally a VL domain, also pluralities and mixtures thereof.

The amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in a fusion with additional amino acids.

As noted, the amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in combination with an antibody VL variable domain thereby forming an antigen-binding site of an antibody.

The present invention further provides a mixture of 10 different specific binding members each comprising an antibody VH variable domain, obtainable from a library as discussed, wherein each antibody VH variable domain has an amino acid sequence selected from the group consisting of the VH domains of Fat3, Fat13, Fat17, Fat31, Fat37, Fat40, Fat86, Fat97, Fat103 and Fat106 (SEQ ID NO.'s being given in Table 5).

In a still further aspect, the present invention further provides a mixture of 10 different specific binding members each comprising an antibody VH variable domain, obtainable from a library as discussed, wherein each antibody VH variable domain has an amino acid sequence comprising a CDR3 selected from the group consisting of the VH domains of Fat3, Fat13, Fat17, Fat31, Fat37, Fat40, Fat86, Fat97, Fat103 and Fat106 (the CDR3 sequences being shown in Table 4).

A composition may be provided in accordance with the present invention to comprise a plurality of different antibody VH variable domains obtainable from such a mixture.

Such a composition may include any one or more of the antibody VH variable domains of Fat3, Fat13, Fat17, Fat31, Fat37, Fat40, Fat86, Fat97, Fat103 and Fat106 (SEQ ID NO.'s being given in Table 5)

Such a composition preferably includes either or both of the antibody VH variable domains of Fat13 and Fat40.

In such a composition, one or more of said antibody VH variable domains may be in a fusion with additional amino acids.

In such a composition, one or more of said antibody VH variable domains may be in association with an antibody VL variable domain, preferably a VL domain disclosed herein.

In any VH/VL domain pairing of VH and VL domains disclosed herein, preferred embodiments include the pairings shown in Table 5.

In a further aspect, the present invention provides an antibody VH variable domain obtainable from a mixture or a

library as disclosed.

Such an antibody VH variable domain may have an amino acid sequence selected from the group consisting of those of Fat3, Fat13, Fat17, Fat31, Fat37, Fat40, Fat86, Fat97, Fat103 and Fat106 (SEQ ID NO.'s being given in Table 5).

A further aspect of the present invention provides nucleic acid, generally isolated, encoding an antibody VH variable domain and/or VL variable domain disclosed herein.

Another aspect of the present invention provides nucleic acid, generally isolated, encoding a VH CDR3 sequence disclosed herein.

A further aspect provides a host cell transformed with such nucleic acid.

A yet further aspect provides a method of production of an antibody VH variable domain, the method including causing expression from encoding nucleic acid. Such a method may comprise culturing host cells under conditions for production of said antibody VH variable domain.

Analogous methods for production of VL variable domains and specific binding members comprising a VH and/or VL domain are provided as further aspects of the present invention.

A method of production may comprise a step of isolation and/or purification of the product.

A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.

Another aspect of the present invention provides a method of obtaining one or more antigen molecules, the method including bringing into contact material suspected of containing an antigen of interest and a specific binding member according to the invention, and selecting one or more antigen molecules bound by said specific binding member. The antigen of interest may, for example, be a specific marker, a molecule involved in fat metabolism, a receptor, a cytokine, an integrin or a signalling molecule.

Such a method may comprise bringing said material into contact with a plurality of specific binding members.

A selected antigen molecule may be provided in an isolated and/or purified form.

A said selected antigen molecule in isolated form may be formulated into a composition including at least one additional component.

A panel, library or mixture of specific binding members provided by the present invention is useful for selection of specific binding members against adipocytes and adipocyte antigens. An antibody panel may for example be used as an immunological tool in techniques such as ELISA, Western blotting, immunocytochemistry, immuno-precipitation and affinity chromatography.

A VH domain of which the sequence is disclosed herein may be combined with a VL domain of which the sequence is disclosed herein, or other VL domain, to provide a VH/VL pairing representing an antigen-binding site of an antibody. Similarly, a VL domain of which the sequence is disclosed herein may be combined with a VH domain of which the sequence is disclosed herein, or other VH domain.

One or more CDRs may be taken from a VH or VL domain and incorporated into a suitable framework. This is discussed further below.

Variants of the VH and VL domains and CDRs of which the sequences are set out herein and which can be employed in specific binding members for adipocytes and adipocyte antigens can be obtained by means of methods of sequence alteration or mutation and screening. Such methods are also provided by the present invention.

Variable domain amino acid sequence variants of any of the VH and VL domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), maybe less than about 20 alterations, less than about 15 alterations, less than about 10 alterations or less than about 5 alterations, 4, 3, 2 or 1. Alterations may be made in one or more framework regions and/or one or more CDR's.

A specific binding member according to the invention may be one which competes for binding to antigen with any specific binding member which both binds the antigen and comprises a specific binding member, VH and/or VL domain disclosed herein, or VH CDR3 disclosed herein, or variant of any of these. Competition between binding members may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

In addition to antibody sequences, the specific binding

member may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Specific binding members of the invention may carry a detectable label, or may be conjugated to a toxin or enzyme (e.g. via a peptidyl bond or linker).

A further aspect of the present invention provides a method of obtaining one or more specific binding members with a desired property, the method including bringing into contact a library or panel of specific binding members and selecting one or more with the desired property. Such a method may employ phage display technology, wherein the specific binding members in the library or panel are displayed on the surface of bacteriophage particles, each particle containing nucleic acid encoding the specific binding member or a component thereof (e.g. VH domain). Nucleic acid may be taken from a bacteriophage particle containing nucleic acid encoding a selected specific binding member or component thereof, and nucleic acid with the sequence of the nucleic acid from the particle can be used to provide (by means of recombinant technology) the encoded product, or further nucleic acid with the sequence, or a variant or derivative.

In further aspects, the invention provides an isolated nucleic acid which comprises a sequence encoding a specific binding member as defined above, and methods of preparing specific binding members of the invention which comprise expressing said nucleic acids under conditions to bring about expression of said binding member, and recovering the binding member.

Specific binding members according to the invention may be used in a method of treatment or diagnosis of the human or animal body, such as a method of treatment (which may include

prophylactic treatment) of a disease or disorder in a human patient which comprises administering to said patient an effective amount of a specific binding member of the invention. Conditions treatable in accordance with the present invention include obesity and obesity related disorders, as disclosed herein.

These and other aspects of the invention are described in further detail below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows results of ELISA experiments with the antibody VH/VL pairing of Fat 37 on various adipocyte plasma membranes from each of the indicated adipose depots.

Figure 2 shows optical densities of various antibody VH/VL pairings measured at different temperatures, as a measure of stability.

TERMINOLOGY

Specific binding member

This describes a member of a pair of molecules which have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. This application is concerned with

antigen-antibody type reactions.

Antibody

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein having a binding domain which is, or is substantially homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to

another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, **242**, 423-426, 1988; Huston et al, PNAS USA, **85**, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al, Proc. Natl. Acad. Sci. USA **90** 6444-6448, 1993). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Y. Reiter et al, Nature Biotech, **14**, 1239-1245, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (S. Hu et al, Cancer Res., **56**, 3055-3061, 1996).

Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site:

antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. **4**, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. It may be preferable to use scFv dimers or diabodies rather than whole antibodies. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E.coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al, Protein Eng., **9**, 616-621, 1996).

Antigen binding domain

This describes the part of an antibody which comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be

provided by one or more antibody variable domains (e.g. a so-called Fd antibody fragment consisting of a VH domain). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

Specific

This may be used to refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s). The term is also applicable where e.g. an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope.

Comprise

This is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

Isolated

This refers to the state in which specific binding members of the invention, or nucleic acid encoding such binding members, will be in accordance with the present invention. Members and nucleic acid will be free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will normally be mixed

with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Specific binding members may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells), or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

By "substantially as set out" it is meant that the relevant CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, substitutions may be made in the CDR and/or VH or VL domain.

The structure for carrying a CDR of the invention will generally be of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR is located at a location corresponding to the CDR of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to (Kabat, E.A. et al, Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (http://immuno.bme.nwu.edu)).

Preferably, a CDR amino acid sequence substantially as set out herein is carried as a CDR in a human variable domain or a substantial portion thereof. The VH CDR3 sequences substantially as set out herein represent preferred embodiments of the present invention and it is preferred that each of these is carried as a VH CDR3 in a human heavy chain

variable domain or a substantial portion thereof.

Variable domains employed in the invention may be derived from any germline or rearranged human variable domain, or may be a synthetic variable domain based on consensus sequences of known human variable domains. A CDR-derived sequence or sequences of the invention (e.g. CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. CDR3), using recombinant DNA technology.

For example, Marks *et al* (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks *et al* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the present invention may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific binding members of the invention. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 so that suitable specific binding members may be selected. A repertoire may consist of from anything from 10^4 individual members upwards, for example from 10^6 to 10^8 or 10^{10} members.

Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (*Nature*, 1994, 370:389-391), who describes the technique in relation to a β-lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions carrying a CDR-derived sequence or sequences of the invention using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram *et al* (1992, *Proc. Natl. Acad. Sci., USA,* 89:3576-3580), who used error-prone PCR.

Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by Barbas *et al*, (1994, *Proc. Natl. Acad. Sci., USA,* 91:3809-3813) and Schier *et al* (1996, *J. Mol. Biol.* 263:551-567).

All the above described techniques are known as such in the art and in themselves do not form part of the present invention. The skilled person will be able to use such techniques to provide specific binding members of the invention using routine methodology in the art.

A further aspect of the invention provides a method for obtaining an antibody antigen binding domain specific for an adipocyte antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, combining the VH domain thus provided with one or more VL domains, and testing the VH/VL combination or combinations for to identify an antibody antigen binding domain specific for an adipocyte antigen and optionally with one or more of preferred properties. Said VL domain may have an amino acid sequence which is substantially as set out herein.

An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are

combined with one or more VH domains.

A further aspect of the invention provides a method of preparing a specific binding member specific for an adipocyte antigen, which method comprises:

(a) providing a starting repertoire of nucleic acids encoding a VH domain which either include a CDR3 to be replaced or lack a CDR3 encoding region;

(b) combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out herein for a VH CDR3 such that said donor nucleic acid is inserted into the CDR3 region in the repertoire, so as to provide a product repertoire of nucleic acids encoding a VH domain;

(c) expressing the nucleic acids of said product repertoire;

(d) selecting a specific binding member specific for an adipocyte antigen; and

(e) recovering said specific binding member or nucleic acid encoding it.

Again, an analogous method may be employed in which a VL CDR3 of the invention is combined with a repertoire of nucleic acids encoding a VL domain which either include a CDR3 to be replaced or lack a CDR3 encoding region.

Similarly, one or more, or all three CDRs may be grafted into a repertoire of VH or VL domains which are then screened for a specific binding member or specific binding members specific for an adipocyte antigen.

A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the

first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of specific binding members of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies) or protein labels as discussed in more details below.

Although in a preferred aspect of the invention specific binding members comprising a pair of VH and VL domains are preferred, single binding domains based on either VH or VL domain sequences form further aspects of the invention. It is known that single immunoglobulin domains, especially VH domains, are capable of binding target antigens in a specific manner.

In the case of either of the single chain specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific binding member able to bind an adipocyte antigen.

This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach as disclosed in WO 92/01047 in which an individual colony containing either an H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H)

and the resulting two-chain specific binding member is selected in accordance with phage display techniques such as those described in that reference. This technique is also disclosed in Marks *et al, ibid.*

Specific binding members of the present invention may further comprise antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to antibody light chain constant domains including human Cκ or Cλ chains, preferably Cλ chains. Similarly, a specific binding member based on a VH domain may be attached at its C-terminal end to all or part of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype sub-classes, particularly IgG1 and IgG4. IgG4 is preferred.

Antibodies of the invention may be labelled with a detectable or functional label. Detectable labels include radiolabels such as ^{131}I or ^{99}Tc, which may be attached to antibodies of the invention using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin.

Antibodies of the present invention are designed to be used in methods of diagnosis or treatment in human or animal subjects, preferably human.

Accordingly, further aspects of the invention provide methods of treatment comprising administration of a specific binding member as provided, pharmaceutical compositions comprising such a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a

medicament or pharmaceutical composition comprising formulating the specific binding member with a pharmaceutically acceptable excipient.

In accordance with the present invention, compositions provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practioners and other medical doctors. Appropriate doses of antibody are well known in the art; see Ledermann J.A. et al. (1991) Int J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Antibodies of the present invention may be administered to a patient in need of treatment via any suitable route, usually by injection into the bloodstream or directly into the site to be treated, e.g. cornea or wound. The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis or for treatment, the size and location of the area to be treated (e.g. wound), the precise nature of the antibody (e.g. whole antibody, fragment or diabody), and the nature of any detectable label or other molecule attached to the antibody. A typical antibody dose will be in the range 0.5mg to 100g for systemic applications, and 10μg to 1mg for local applications. Typically, the

antibody will be a whole antibody, preferably the IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician.

It is presently preferred that a whole antibody of the IgG4 isotype is used for systemic and local applications but for local applications a scFv antibody may be particularly valuable.

Specific binding members of the present invention will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the specific binding member.

Thus pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline

solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Other treatments may include the administration of suitable doses of pain relief drugs such as non-steroidal anti-inflammatory drugs (e.g. asprin, paracetamol, ibuprofen or ketoprofen) or opitates such as morphine, or anti-emetics.

The present invention provides a method comprising causing or allowing binding of a specific binding member as provided herein to an adipocyte antigen. As noted, such binding may take place *in vivo*, e.g. following administration of a specific binding member, or nucleic acid encoding a specific binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation or affinity chromatography.

The amount of binding of specific binding member to an adipocyte antigen may be determined. Quantitation may be related to the amount of the antigen in a test sample, which

may be of diagnostic interest.

The reactivities of antibodies on a sample may be determined by any appropriate means. Radioimmunoassay (RIA) is one possibility. Radioactive labelled antigen is mixed with unlabelled antigen (the test sample) and allowed to bind to the antibody. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the antibody determined. The more antigen there is in the test sample the less radioactive antigen will bind to the antibody. A competitive binding assay may also be used with non-radioactive antigen, using antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The signals generated by individual antibody-reporter

conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

The present invention also provides the use of a specific binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a specific binding member as provided by the present invention in a competition assay. This may be where the physical separation of bound from unbound antigen is not required. Linking a reporter molecule to the specific binding member so that a physical or optical change occurs on binding is one possibility. The reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

The present invention also provides for measuring levels of antigen directly, by employing a specific binding member according to the invention for example in a biosensor system.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

As noted, an adipocyte antigen may be on the surface of an adipocyte. Accordingly, methods of detection and determination of the presence or level of adipocyte antigen, and other methods and uses herein, encompass such methods when used to detect or determine the presence or level of

adipocytes, for example in a cell or tissue sample, except where context requires otherwise.

The present invention further extends to a specific binding member which competes for binding to an adipocyte antigen with any specific binding member which both binds the antigen and comprises a V domain including a CDR with amino acid sequence substantially as set out herein or a V domain with amino acid sequence substantially as set out herein. Competition between binding members may be assayed easily *in vitro*, for example by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope. Competition may be determined for example using the ELISA as described in Example 5 or Example 7.

In testing for competition a peptide fragment of the antigen may be employed, especially a peptide including an epitope of interest. A peptide may have the epitope sequence plus one or more amino acids at either end, may be used. Such a peptide may be said to "consist essentially" of the specified sequence. Specific binding members according to the present invention may be such that their binding for antigen is inhibited by a peptide with or including the sequence given. In testing for this, a peptide with either sequence plus one or more amino acids may be used.

Specific binding members which bind a specific peptide may be isolated for example from a phage display library by panning with the peptide(s).

The present invention further provides an isolated nucleic acid encoding a specific binding member of the present

invention. Nucleic acid includes DNA and RNA. In a preferred aspect, the present invention provides a nucleic acid which codes for a CDR or VH or VL domain of the invention as defined above.

The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

The present invention also provides a recombinant host cell which comprises one or more constructs as above. A nucleic acid encoding any CDR, VH or VL domain, or specific binding member as provided itself forms an aspect of the present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression of a VH or VL domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

Specific binding members, VH and/or VL domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires

otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Plückthun, A. Bio/Technology **9**: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding member, see for recent reviews, for example Ref, M.E. (1993) Curr. Opinion Biotech. **4**: 573-576; Trill J.J. et al. (1995) Curr. Opinion Biotech **6**: 553-560.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*,

Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

The present invention also provides a method which comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as above.

Aspects and embodiments of the present invention will now be illustrated by way of example with reference to the following experimentation.

LIST OF EXAMPLES

EXAMPLE 1: Preparation Of Adipocytes From Human Adipose Tissue And Isolation Of A Plasma Membrane Rich Fraction.

EXAMPLE 2: Selection Of Antibody-Expressing Phage by Panning on Isolated Adipocytes and Adipocyte Plasma Membranes.

EXAMPLE 3: Selection Of Antibody-Expressing Phage by Panning on Intact Adipose Tissue.

EXAMPLE 4: ProxiMol Selection Of Antibody-Expressing Phage Using An Anti-Adipocyte Polyclonal Serum As The Targeting Reagent.

EXAMPLE 5: Identification & Characterisation Of Selected Antibodies By Adipocyte Plasma Membrane ELISA And DNA Sequencing.

EXAMPLE 6: Characterisation Of Anti-Adipocyte Antibodies By Immunocytochemistry On Human Tissues.

EXAMPLE 7: Characterisation Of Antigens Recognised By The Anti-Adipocyte Antibodies.

ABBREVIATIONS

Immunocytochemistry (ICC)
Enzyme linked immunosorbent assay (ELISA)
Bovine serum albumin (BSA)
Horseradish peroxidase (HRP)
Bacterial growth medium (2TY: 16g Bacto-tryptone, 10g Yeast extract and 5g NaCl per litre of distilled water)
Bacterial growth medium (2TYAG: 2TY supplemented with 100mg/ml ampicillin and 2% glucose)

Bacterial growth medium (2TYAK: 2TY supplemented with 100mg/ml ampicillin and 50mg/ml kanamycin)
Phosphate Buffered Saline (PBS)
Phosphate Buffered Saline + 0.1% (v/v) Tween 20 (PBST)
Triethylamine (TEA)
Multiplicity of Infection (MOI)
Immobilised Metal Affinity Chromatography (IMAC)
Polyacrylamide Gel Electrophoresis (PAGE)
Isoelectric Focusing (IEF)
Polymerase Chain Reaction (PCR)
Polyvinylidene Difluoride (PVDF)
3,3',5,5'-Tetramethyl Benzidine (TMB)
N-Hydroxysuccinimide (NHS)
Critical micelle concentration (CMC)
Sodium dodecyl sulphate (SDS)
Surface-enhanced laser desorption/ionization (SELDI mass spectrometry)

EXAMPLE 1: PREPARATION OF ADIPOCYTES FROM HUMAN ADIPOSE TISSUE AND ISOLATION OF A PLASMA MEMBRANE RICH FRACTION

1.1 Sources of adipose tissue.

Normal human adipose tissue was obtained from a number of depots from both male & female patients, aged between 24-79: breast, abdomen, thigh, omental (also known as visceral or intraperitoneal adipose), back, arm, eye and lipoma (fatty tumour tissue). Depending on the nature of the surgery involved, the amount of fat obtained ranged from a couple of grams to over 1kg. All adipose samples were treated in an identical manner.

1.2 Preparation of adipocytes.

Adipocytes were prepared from intact adipose as described by Kestin (J. Anim. Sci., 71, 1486-94, 1993). Up to 100g of adipose tissue was minced with scissors until the pieces were

less than 5mm^3 in size. This minced adipose tissue was then mixed with prewarmed (37°C) medium 199 (2 vols/wt), 3% BSA (w/v) (Fatty acid free, Boehringer 775835) and 1mg/ml collagenase (Sigma C-6885) and incubated at 37°C with shaking at 120rpm for between 30-60 mins. The reaction was deemed complete when the majority of the adipose tissue pieces had been digested and a layer of adipocytes could be seen floating on the surface of the medium.

To remove undigested tissue, the reaction mix was diluted 5:1 (v/v) with prewarmed medium 199 and sieved (0.25µm pore size) into a fresh beaker. Viable adipocytes were allowed to float to the surface and the medium aspirated from beneath the cells. A further 3 volumes of prewarmed medium 199 was added to the cells which were resuspended by gentle swirling. Adipocytes were once again allowed to float and the medium removed from beneath the cells. This washing procedure was then continued until the wash media beneath the cells appeared clear, usually after 3-5 washes. Cells were then taken directly for selections (see Example 2), otherwise unused cells were mixed (1:1 v/v) with 12mM Tris, 0.25M sucrose, pH 7.5 and stored frozen at -70°C for preparation of plasma membranes.

1.3 Preparation of a plasma membrane rich fraction from adipocytes

Adipocytes frozen at -70°C in 12mM Tris, 0.25M sucrose, pH 7.5 were thawed to room temperature. To ensure complete cell lysis, the adipocytes were once again frozen in dry ice and thawed to room temperature. The adipocytes were then centrifuged at 3000rpm for 2 minutes at room temperature. Following this step, the lysed adipocytes will partition in a test tube with a pellet of ruptured cells and cell debris at the bottom, under a layer of infranatant above which is a layer of intact adipocytes under a layer of clear lipid.

Tubes were placed on ice until the lipid layer had solidified. The infranatant, which contains the plasma membrane fraction, was pipetted into a fresh tube and then subjected to a series of centrifugations at 4°C. A nuclear fraction was recovered at 270xg for 10 minutes and the supernatant centrifuged for 10 minutes at 8000xg for the recovery of a mitochondrial and lysosomal fraction. The plasma membrane fraction was subsequently recovered by centrifugation at 104,000xg for 60 minutes from the post mitochondrial supernatant, resuspended in PBS and frozen at -70°C until used. Total protein concentration of the membrane fraction was determined using the Bradford assay reagent (Pierce 500-0006).

1.4 Summary

Human adipose tissue was obtained from a number of depots. Isolation of adipocytes from these tissues was performed rapidly, with viable cells being identified as the population of cells floating at the surface of the tissue homogenate. Once isolated, the floating adipocytes pack together at approximately $2x10^6$ cells/ml and from 100g of adipose approximately 500mls or $1x10^9$ adipocytes could be isolated. These adipocytes were extensively washed and 50µl or $1x10^5$ cells used directly in selections (see Example 2). The remaining adipocytes were used to prepare plasma membranes. For a typical preparation, 50ml or $1x10^8$ adipocytes yielded around 1mg of plasma membranes in 0.5ml PBS. These plasma membranes were then used in further selections or for screening the anti-adipocyte antibodies by ELISA.

EXAMPLE 2: SELECTION OF ANTIBODY-EXPRESSING PHAGE BY PANNING ON ISOLATED ADIPOCYTES AND ADIPOCYTE PLASMA MEMBRANES

2.1 Antibody repertoire

A Large single chain Fv library derived from lymphoid tissues

including tonsil, bone marrow and peripheral blood lymphocytes (Vaughan et al, Nature Biotechnology, 14, 309-314, 1996) was used for all selections.

Polyadenylated RNA was prepared from the B-cells of various lymphoid tissues of 43 non-immunised donors using the "Quickprep mRNA Kit" (Pharmacia). First-strand cDNA was synthesized from mRNA using a "First-strand cDNA synthesis" kit (Pharmacia) using random hexamers to prime synthesis. V-genes were amplified using family-specific primers for VH, Vλ and Vκ genes as previously described (Marks et al., (1991) J. Mol. Biol. 222:581-597) and subsequently recombined together with the $(Gly_4, Ser)_3$ scFv linker by PCR assembly. The VH-linker-VL antibody constructs were cloned into the Sfi I and Not I sites of the phagemid vector, pCANTAB6. Ligation, electroporation and plating out of the cells was as described previously (Marks et al, supra). The library was made ca. 1000x larger than that described previously by bulking up the amounts of vector and insert used and by performing multiple electroporations. This generated a scFv repertoire that was calculated to have ca. 1.3×10^{10} individual recombinants which by Bst NI fingerprinting were shown to be extremely diverse.

2.2 Induction of phage antibody library to produce phage particles

The phage antibody repertoire above was selected for antibodies which bind to adipocytes. The 'large' scFv repertoire was treated as follows in order to rescue phagemid particles. 500ml prewarmed (37°C) 2YTAG (2YT media supplemented with 100µg/ml ampicillin and 2% glucose) in a 2l conical flask was inoculated with approximately $3x10^{10}$ cells from a glycerol stock (-70°C) culture of the library. The culture was grown at 37°C with good aeration until the OD_{600nm} reached 0.7 (approximately 2 hours). M13K07 helper phage

(Stratagene) was added to the culture to a multiplicity of infection (moi) of approximately 10 (assuming that an OD_{600nm} of 1 is equivalent to 5×10^8 cells per ml of culture). The culture was incubated stationary at 37°C for 30 minutes followed by 30 minutes with light aeration (200rpm) at the same temperature. The culture was centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 500ml 2YTAK (2YT media supplemented with 100µg/ml ampicillin and 50µg/ml kanamycin), and the culture incubated overnight at 30°C with good aeration (300rpm). Phage particles were purified and concentrated by one polyethylene glycol (PEG) precipitation (Sambrook, J., Fritsch, E.F., & Maniatis, T. (1990). Molecular Cloning - A Laboratory Manual. Cold Spring Harbour, New York) and resuspended in 9ml 10mM Tris containing 1mM EDTA (TE). 4.0g of CsCl was added to the phage stock and mixed gently to dissolve. A 11.5ml ultracentrifuge tube was filled with phage and centrifuged at 40 000rpm at 25°C for 24hr. The ultracentrifuge was stopped with the brake off and the clear opalescent phage band collected using a pasteur pipette. Phage were dialysed at 4°C overnight against two changes of 1l of TE, titred and stored at 4°C.

2.3 Selection of phage from the large phage library which bind to adipocytes

Selections were performed on either freshly isolated adipocytes or on plasma membrane fractions. In addition, the adipose tissue used in the selections was obtained from a number of different locations, i.e. subcutaneous, omental and lipoma depots. By varying the types of selections performed and the adipose source we aimed to maximise the diversity of antibodies that were obtained and the range of antigens that they bind.

2.3.1 First round of selection

Freshly isolated adipocytes, but not plasma membrane fractions, were used for the first round of selection. This was to ensure that antibodies to the adipocyte cell surface only were selected at the first round, since selection on plasma membranes could yield antibodies binding only to intracellular antigens as well as those binding only to cell surface antigens. For each selection 50µl of isolated adipocytes were used, which is approximately $1x10^5$ cells. These cells were prepared for selection by washing once in 250µl of Dulbeccos PBS plus 1% fatty acid free BSA. The washed adipocytes were then centrifuged for 1 minute at 1000rpm in a microfuge and the PBS removed from beneath the floating adipocytes.

Prior to selection, $1x10^{13}$ phage from the above library were preblocked in 250µl of 1% fatty acid free BSA, Dulbeccos PBS for 30 minutes at room temperature. The preblocked phage were then added directly to the freshly isolated adipocytes and incubated for 1 hour at 37°C with a single inversion every 15 minutes to mix. The adipocytes were then washed 3 times in Dulbeccos PBS plus 0.1% Tween 20 followed by 3 washes in Dulbeccos PBS. Each wash consisted of resuspending the cells in 250µl of wash buffer, followed by microfuging for 1 minute at 1000rpm and pipetting off the supernatant from beneath the adipocytes.

Elution of bound phage was achieved by mixing the adipocytes directly with 10ml of exponentially growing E coli TG1 with light aeration in 2TY broth at 37°C for 1 hour. Infected TG1's were plated on 2TYAG medium in 243mm x 243mm dishes. Dilutions of infected TG1s were also plated out and incubated at 30°C overnight. Colony counts gave the phage output titre.

2.3.2 Second round of selection

For the second round of selection, the round one outputs were

panned either on (1) freshly isolated adipocytes a second time, or (2) on an adipocyte plasma membrane preparation. Freshly isolated adipocytes were prepared for selections in the same way as described for round 1. The adipose tissue or plasma membrane preparation used was also consistent with that used for round 1, i.e. if the adipose depot used at round one was subcutaneous then the same depot was used for round two. For selections on adipocyte plasma membranes, plasma membranes were coated onto immunotubes (Nunc) at 10mg/ml in 1ml of PBS overnight at 4°C. Uncoated material was washed away using PBS and the immunotube blocked with 1% fatty acid free BSA in PBS for 60 minutes at 37°C.

From the round one selections, colonies were scraped off the 243mm x 243mm plates into 3 ml of 2TY broth and 25% (v/v) glycerol added for storage at -70°C. Glycerol stock solutions from the first round of selection were rescued using helper phage to derive phagemid particles for the second round of selection. 250µl of a glycerol stock was used to inoculate 50 ml 2YTAG broth, and incubated in a 250 ml conical flask at 37°C with good aeration until the OD_{600nM} reached 0.7 (approximately 2 hours). M13K07 helper phage (moi=10) was added to the culture which was then incubated stationary at 37°C for 30 minutes followed by 30 minutes with light aeration (200rpm) at the same temperature. The culture was centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 50ml prewarmed 2YTAK, and the culture incubated overnight at 30°C with good aeration. Phage particles were then obtained by centrifuging the overnight culture at 13000rpm in a microfuge for 2 minutes. The supernatant, which contains approximately 1×10^{12} phage per ml, was decanted into a fresh tube and the centrifugation repeated.

For the second round of selection using freshly isolated

adipocytes, 1ml of phage supernatant was concentrated by addition of 300µl 20%(w/v) PEG, 2.5M NaCl. The phage were precipitated for 1 hour on ice and recovered by microfuging for 10 minutes at 13000rpm. The supernatant was discarded and the phage pellet resuspended in 250µl of Dulbecco's PBS containing 1% fatty acid free BSA. 1µl of this PEG precipitated phage, containing approximately 4 x 10^9 phage particles (assuming that an overnight rescue produces a phage titre of 1 x 10^{12} phage/ml), was diluted 1:400000 and 1µl (1 x 10^4 phage) reinfected into *E.coli* to determine the actual input titre. The remaining preblocked phage (approximately 1 x 10^{12} phage particles) was then added directly to the cells and incubated for 1 hour at 37°C. As with round one, the adipocytes were inverted once every 15 minutes during this binding step. The washing and elution steps were also identical to those described for round one.

For the selections on adipocyte plasma membranes, phage supernatants were preblocked in 1% fatty acid free BSA and 1xPBS for 30 minutes at room temperature. 1ml of preblocked phage was added directly to the plasma membrane coated immunotube and left stationary for 1 hour at 37°C. The tubes were then washed ten times with PBST and ten times with PBS. Phage were eluted by the addition of 100mM TEA for 10 minutes at room temperature. The TEA was then neutralised with ½ volume of 1M Tris.Cl, pH 7.6 and the eluted phage infected into exponentially growing TG1.

TG1 infection, plating out and titring was performed as described for round 1.

2.3.3 Third round of selection

The outputs from the second round of selection were scraped off the large plates and phage rescued for a third round of selection. The methods followed in the third round of

selection were identical to those described for round two. Briefly, selections were performed on either freshly isolated adipocytes or adipocyte plasma membranes and the choice of selection followed on directly from those performed at rounds 1 and 2. A summary of each type of selection performed (rounds 1 to 3) along with the output titres obtained is shown in Table 1.

2.4 Summary

The selection strategy was designed so that a large panel of anti-adipocyte antibodies binding several different antigens would be isolated from the large human scFv library. It was expected that the antibody-antigen interaction would be driven by the density of any given antigen on the adipocyte cell surface. To maximise the diversity of antibodies produced, selections were performed using adipocytes from different depots, i.e. from either subcutaneous, omental or lipoma adipose tissue, as the nature of the antigens themselves or their cell surface density may vary between depots. By selecting the antibody library on adipocytes from different adipose depots it was aimed to maximise the diversity of the clone panel obtained and to possibly isolate antibodies specific for each depot.

For each round of selection, freshly isolated adipocytes were used to ensure that antibodies subsequently isolated recognised antigens on the adipocyte cell surface. Generally the adipocytes were difficult to handle because (a) they float, and (b) they lyse if shaken too vigorously. Despite these technical difficulties, the adipocytes were sufficiently stable to survive the selection and subsequent washing procedures. As an alternative approach, adipocyte plasma membranes were also used as part of the selection strategy as they are relatively easy to handle. However, to avoid the isolation of antibodies to intracellular antigens,

selections were only performed on isolated adipocytes and not plasma membranes at round one. By avoiding the selection of antibodies to intracellular epitopes at round one (when the library has greatest diversity), only antibodies binding to adipocyte cell surface antigens will be subsequently enriched at rounds 2 and 3. Therefore, plasma membranes were only used as an alternative to freshly isolated adipoctes in the second and third rounds of selection.

For each type of selection, three rounds of panning were performed. After each round of selection, ELISA analysis demonstrated that the number of clones binding to adipocyte plasma membranes increased from rounds one to three. This observation demonstrated that the number of antibodies that recognised the adipocyte cell surface was being enriched with each round of selection even though the total output for each round did not vary a great deal. The anti-adipocyte antibodies isolated from these selections were then assessed by phage ELISA for adipocyte specificity (see Example 5).

EXAMPLE 3: SELECTION OF ANTIBODY-EXPRESSING PHAGE BY PANNING ON INTACT ADIPOSE TISSUE.

As well as panning the phage antibody library on freshly isolated adipocytes and plasma membranes, selections were also carried out on intact adipose. These selections used the same antibody repertoire as described in Example 2.

3.1 Selections on intact adipose at different temperatures

Selections on intact adipose tissue were performed as an alternative to using freshly isolated adipocytes. The disadvantage of taking this approach is that there is a range of cell types other than adipocytes present in adipose that will bind antibodies from the library, e.g. pre-adipocytes or vascular endothelial cells. However, the principal benefit of

selecting on intact adipose is that collagenase will not have been employed. Collagenase (which contains trace amounts of other proteases) may potentially destroy some of the antigens of interest on the adipocyte cell surface during the digestion step detailed in section 1.2.

In addition to selecting on intact adipose, the effect of varying temperature was also investigated. Selections are typically performed at 37°C, but at this temperature cell surface receptors may be rapidly internalised (Walker F, J. Cell. Physiol., 130, 255, 1987). Therefore, selections were also performed at lower temperatures (4°C and room temperature) to minimize this effect.

Adipose tissue was prepared for selection by mincing into very small pieces (approximately 2-3mm thick) with scissors followed by washing in Dulbeccos PBS. One round of selection was then performed on these adipose tissue pieces as described in Example 2, with the exception that the incubation temperatures used were 4°C and room temperature. Rounds two and three were performed only on adipocyte plasma membranes as described in Example 2, once again at 4°C and room temperature.

A summary of these selections and the output titres is given in Table 2.

3.2 Summary

Selections were performed on minced adipose tissue to isolate antibodies to collagenase sensitive antigens/epitopes. By selecting the antibody library on intact tissue, antibodies would also be isolated to other antigens present in adipose, e.g. extracellular matrix antigens like collagen or antigens expressed by other cell types such as pre-adipocytes or vascular endothelial cells. However, to prevent enrichment

of antibodies binding to antigens on cell types other than adipocytes, rounds 2 and 3 were performed on adipocyte plasma membranes to select for adipocyte binding clones only.

The output titres for selections at both 4°C and 25°C shared similar trends. High output titres were observed after the first round of selection, at least 10-100 fold above those seen for the selections on isolated adipocytes (see Example 2). These higher titres most likely reflect that intact tissue has been used for selections rather than a single cell type such as an adipocyte. These antibodies were then assessed by phage ELISA for adipocyte specificity (see Example 5).

EXAMPLE 4: PROXIMOL SELECTION OF ANTIBODY-EXPRESSING PHAGE USING AN ANTI-ADIPOCYTE POLYCLONAL SERUM AS THE TARGETING REAGENT

4.1 ProxiMol Selections using an anti-adipocyte polyclonal serum

It is possible to target phage antibody libraries to cell surface proteins by using targeting molecules such as their natural ligands or specific antibodies to the proteins of interest (monoclonal or polyclonal). Here, a rabbit polyclonal anti-adipocyte serum was employed to guide phage antibodies to proteins on the adipocyte cell surface using the method described by Osbourn et al (Immunotechnology, 3, 293, 1998, and WO98/01757).

4.2 Generation and Characterisation of the Rabbit Anti-Adipocyte Polyclonal Serum

To generate antibodies against human adipocyte plasma membranes, rabbits were immunised 4 times with 100µg human adipocyte plasma membranes at 28 day intervals and serum

samples taken. The serum was characterised by ELISA and shown to bind human adipocyte plasma membranes at dilutions down to 1:100000. However, the serum is not specific for adipocytes and cross-reaction was observed to plasma membrane preparations from other cell lines, e.g. Chang liver hepatocytes, lung fibroblast cell line CCD-19Lu, macrophage like cell line U937 and human umbilical cord endothelial cell line HuVEC. However, this cross-reaction is not observed at serum concentrations below 1:1000, so the serum is relatively specific for adipocyte antigens.

4.3 ProxiMol selection using the anti-adipocyte rabbit polyclonal serum as a targetting agent

The antibody library described in example 2 was also used in these selections. To prepare the rabbit polyclonal serum for ProxiMol, it was first necessary to conjugate it to HRP using an HRP conjugation kit (Pierce 31494). ELISA was employed to determine that the HRP conjugation was successful and to empirically determine the optimal HRP-polyclonal concentration to use in ProxiMol. Adipocyte plasma membranes (10µg/ml in PBS) were coated overnight at 4°C onto 96 well polystyrene plates (Falcon 3912). Uncoated material was washed away using PBS and each well blocked with 200ml of 3% Marvel in PBS for 1 hour at 37°C. A serial dilution (1:10, 1:100 and 1:1000) of the HRP conjugated anti-adipocyte serum in 3% Marvel/PBS was then applied to the plasma membrane coated wells of a 96 well plate and incubated for 1hour at 37°C. Unbound antibody was washed away with 3 PBST washes followed by 3 PBS washes. Bound antibody was then detected with TMB substrate (Sigma T-8665). Colour was allowed to develop to a suitable intensity, the reaction was then stopped by the addition of ½ volume 0.5M H_2SO_4 and the absorbance of each well measured at 450nm. In this ELISA the polyclonal-HRP conjugate was shown to bind effectively to adipocyte but not hepatocyte plasma membranes at a dilution

of 1:100.

The first round of ProxiMol selection was conducted as described in Example 2, i.e adipocytes were freshly prepared and incubated together with the large scFv library. However, for a ProxiMol selection, the anti-human adipocyte polyclonal conjugated to HRP was simultaneously added to a final concentration of 1:100. Parallel selections were set up, one incubated at 4°C and the other at room temperature (25°C). For both selections, the incubation time was two hours and the tubes were inverted once every 20 minutes to ensure mixing. The adipocytes were then washed 3x with PBST and 3x with PBS. The selections were then treated with biotin tyramine. In the presence of HRP and hydrogen peroxide biotin tyramine becomes free radicalised and is deposited around the site of HRP activity. Because the half life of the free radical is very short, deposition is highly localised around the site of enzyme activity and only phage which bind close to the site of the original HRP binding site and hence the target antigen, become biotinylated. These phage can be specifically recovered from the background population of non-biotinylated phage.

Biotin tyramine treatment consisted of incubating the selections for 10 min at room temperature with 250µl of biotin tyramine mix containing 0.03% hydrogen peroxide in 50mM Tris-HCl pH 7.4 with a 1:600 dilution of biotin tyramine. Biotin tyramine was prepared by the addition of 5mg of NHS-LC-biotin to 1.55mg tyramine in 2ml 50mM borate, pH 8.8 followed by turning end over end at room temperature in the dark, then filtering through a 45mm filter. The biotin tyramine was aliquotted and stored at -70°C. After the 10 min incubation with biotin tyramine mix the adipocytes were washed in PBST and PBS as before and 500µl of 100mM TEA added to elute bound phage. Incubation was for 10 min at room

temperature, after which the adipocytes were centrifuged for 1 minute at 1000rpm and the eluted phage transferred from beneath the adipocyte layer into a fresh tube containing 250µl of 1M Tris pH7.4.

Eluted phage were added to 50µl of streptavidin coated Dynal beads which had been pre-blocked in PBS containing 3% Marvel (3%MPBS). Phage were incubated with the beads for 15 min at room temperature with end over end rotation in the presence of 3%MPBS. Beads were then washed three times in 1ml of PBST, transferred to a fresh eppendorf and washed three times in 1ml PBS. Finally the beads were resuspended in 100µl of PBS and 50µl of this used to infect TG1 *E.coli* as described in Example 2.

Phage from the first round of selection were rescued as described in Example 2, and a second round of ProxiMol selection carried out exactly as the first, except that both freshly isolated adipocytes and adipocyte plasma membranes were used. The temperature of the second round of selection remained consistent with the first, i.e. a 4°C incubation at round 1 was followed by another 4°C incubation at round 2.

A summary of the ProxiMol selections and the output titres is given in Table 3.

4.4 Summary

The majority of selections have involved panning on either freshly isolated adipocytes, intact adipose tissue or adipocyte plasma membranes. By panning the antibody library on whole cells in this way the inventors expected the selection outputs to be largely driven by antigen density, i.e. the inventors expected antibodies binding to the more abundant antigens on the adipocyte cell surface and fewer antibodies binding to less abundant antigens.

With the aim of deriving a panel of antibodies with different specificities to those obtained by direct panning, ProxiMol selections were also performed, these allowing for the antibody library to be selected in a more targeted manner using, for example, natural ligands or antibodies. In this instance, the inventors opted to use a rabbit polyclonal antiserum that had been raised against human adipocyte plasma membranes. The antibodies in this serum were used to guide the phage antibody library to antigens on the adipocyte cell surface that were originally immunogenic in the rabbit.

The ProxiMol selections were performed at either 4°C or 25°C to avoid possible receptor internalisation and the outputs observed were lower than those seen for direct panning. This is as expected since a more targeted approach yields fewer antibodies than would selections on an entire cell surface. The antibodies from these selections were then assessed by phage ELISA for adipocyte specificity (see Example 5).

EXAMPLE 5: IDENTIFICATION & CHARACTERISATION OF SELECTED ANTIBODIES BY ADIPOCYTE PLASMA MEMBRANE ELISA AND DNA SEQUENCING

5.1 Adipocyte plasma membrane ELISA

With the aim of identifying clones from the selections that bind to adipocyte cell surface antigens, phage antibodies were initially screened by ELISA against both adipocyte and Chang liver hepatocyte plasma membranes. This ELISA would allow us to identify antibodies that recognise the adipocyte cell surface and simultaneously eliminate those antibodies binding common house-keeping proteins present on other cell types.

Phage ELISAs were carried out as follows: individual colonies were picked into a 96 well tissue culture plate containing

100µl 2YTAG. Plates were incubated at 37°C for 6 hours. M13KO7 helper phage was added to each well to an moi of 10 and incubated for 30 min at 37°C followed by gentle shaking for 30min at 37°C. The plates were centrifuged at 2000 rpm for 10min and the supernatant removed. Cell pellets were resuspended in 100µl 2TYAK and incubated at 30°C overnight. Each plate was centrifuged at 2000 rpm and the 100µl phage-containing supernatant from each well recovered and 20µl of 6x PBS containing 18% Marvel™ blocking solution added and then incubated at room temperature for 1 hour. Meanwhile, Falcon™ 96 well polystyrene plates, coated overnight with adipocyte and hepatocyte plasma membranes at 10µg/ml in PBS, were blocked for 2 h at room temperature in PBS containing 3% Marvel (3MPBS). These plates were then washed three times with PBS and 50µl preblocked phage added to each well. The plates were incubated stationary at room temperature for 1 h after which the phage were flicked out. The plates were washed with three changes of PBST followed by three changes of PBS at room temperature.

To each ELISA plate well, 50µl of a 1 in 5000 dilution of the anti-gene8-HRP conjugate (Pharmacia) in 3MPBS was added and the plates incubated at room temperature for 1 h. Each plate was washed with 3xPBST followed by 3xPBS. 50µl of TMB substrate was then added to each well, and incubated at room temperature for approximately 30 minutes, after which the colour reaction was stopped by the addition of 25µl of 0.5MH2SO4. The absorbance signal generated by each clone was assessed by measuring the optical density at 450nm using a microtitre plate reader. Clones were chosen for further analysis if an ELISA signal was observed on adipocyte but not hepatocyte plasma membranes. Of 4400 clones screened from the above selections, over 800 were scored positive by this adipocyte phage ELISA that did not recognise the liver hepatocyte cell line plasma membranes. Thus, over 800 phage

antibodies were identified to antigens present on adipocytes that are not on liver hepatocytes.

5.2 DNA Sequencing of anti-adipocyte antibodies

The nucleotide sequences of the adipocyte binding antibodies were determined by first using vector-specific primers to amplify the inserted DNA from each clone. Cells from an individual colony on a 2YTAG agar plate were used as the template for a PCR amplification of the inserted DNA using the primers pUC19reverse and fdtetseq (Osbourn et al, J. Immunotechnology, 2, 181-96, 1996). Amplification conditions consisted of 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2min, followed by 10 min at 72°C. The PCR products were purified using a PCR Clean-up Kit (Promega) in to a final volume of 50µl H_2O. 5 µl of each insert preparation was used as the template for sequencing using the Taq Dye-terminator cycle sequencing system (Applied Biosystems). The primers pUC19reverse and fdtseq were used to sequence the heavy and light chain of each clone respectively.

The VH and VL segments of the anti-adipocyte clones were then DNA sequenced. From this data we were able to identify 200 unique antibodies for further analysis.

5.3 Specificity ELISA

In order to focus on antibodies potentially binding to novel adipocyte cell surface proteins, a more extensive screen was then performed with the aim of identifying and excluding anti-adipocyte antibodies that were recognising common cell surface or house-keeping proteins expressed by a number of different cell types. The 200 unique antibodies were analysed by phage ELISA for binding to adipocyte plasma membranes but not to plasma membranes from one or more of liver hepatocytes (Chang), erythrocytes, lung fibroblasts (CCD-19Lu), macrophage like cells (U937) and human umbilical cord

endothelial cells (HuVEC).

Individual clones were picked into 50ml Falcon tubes containing 5ml 2YTAG. The tubes were incubated at 37°C for 6 hours. M13KO7 helper phage was added to each tube to an moi of 10 and incubated for 30 min at 37°C followed by gentle shaking for 30min at 37°C. The tubes were centrifuged at 3000 rpm for 10min and the supernatant removed. Cell pellets were resuspended in 5ml 2TYA with kanamycin (50µg/ml) and incubated at 30°C overnight. Each tube was centrifuged at 3000 rpm and 100µl of phage-containing supernatant recovered and blocked with the addition of 20µl 6x PBS containing 18% Marvel™ at room temperature for 1 hour. The phage ELISAs were then performed as described above, with all the plasma membrane preparations being coated overnight at 10µg/ml.

From this additional specificity ELISA, 108 clones were identified that bound to adipocyte but not to hepatocyte, erythrocyte, lung fibroblast, Huvec or U937 plasma membranes. Each of these antibodies is unique as determined by DNA sequencing (see 5.2 above). The sequences are provided below. Note that a number of the antibodies share light chains in common: the sequences for these are not duplicated but are cross-referenced to each other. Table 5 shows the SEQ ID NO.'s for the VH and VL domains. The individual VH and VL segments of the antibodies were aligned to the germline sequences in V-base (Tomlinson et al, MRC centre for Protein Engineering, http://www.mrc-cpe.cam.ac.uk) and the closest germline identified. The result of the V-base search is shown in Table 4, together with the VH CDR3 sequence as a further indication of antibody diversity.

5.4 Adipocyte depot ELISA

As described in Examples 2-4, selections were performed on adipocytes isolated from different body depots. To determine

if any of the antibodies isolated were specific for a particular type of adipose tissue, an ELISA was performed using adipocyte plasma membranes isolated from different depots, i.e. abdominal fat, breast fat, back fat, omental (intra-abdominal) fat and lipoma fat.

All the clones tested in this ELISA were shown to bind to adipocyte plasma membranes from each of the adipose depots. For example, see Figure 1 for results obtained for Fat 37.

As shown in Figure 1, in ELISA Fat 37 is seen to bind adipocyte plasma membranes from each of the fat depots investigated.

5.5 Summary

From the selections (see Examples 2 to 4), a total of 4400 clones were initially screened for specificity by ELISA on both adipocyte and hepatocyte plasma membranes. This preliminary ELISA identified 800 anti-adipocyte antibodies that did not cross-react with liver hepatocytes. These clones were then DNA sequenced and 200 unique anti-adipocyte antibodies identified.

These 200 clones were then screened extensively by ELISA to eliminate those clones binding to one or more of a number of cell types: liver hepatocytes (Chang), erythrocytes, lung fibroblasts (CCD-19Lu), macrophage like cells (U937) and human umbilical cord endothelial cells (HuVEC). The aim of this screen was to identify those clones with the highest specificity for adipocytes and eliminate those that are binding to common house-keeping proteins expressed by a number of cell types. Of the selections described in Examples 2 to 4, those performed using intact adipose tissue generated the highest proportion of antibodies showing cross-reaction with other cell types. In contrast, relatively few

cross-reactive clones were identified in either the adipocyte panning selections or the ProxiMol™ selection. Taken together, these results demonstrate that the likelihood of isolating an antibody with high specificity for a given cell type is increased if that cell is first isolated away from surrounding cell types present in the tissue. Of the 200 clones screened in this specificity ELISA, 108 were identified as adipocyte specific. Each of these antibodies was then further characterised by ICC on normal human tissues (Example 6).

EXAMPLE 6: CHARACTERISATION OF ANTI-ADIPOCYTE ANTIBODIES BY IMMUNOCYTOCHEMISTRY ON HUMAN TISSUES

The clones identified as adipocyte reactive in ELISA (Example 5) were then analysed further by ICC.

In the first instance all antibodies were screened on a tissue rich in adipocytes, such as breast, to confirm that each antibody recognised native adipocytes *in situ*. Antibodies which bound adipose tissue sections were then screened on all (or a subset) of the following panel of normal human tissues: adrenal gland, aorta, bladder, blood vessels, bone marrow, breast, cerebrum, cerebellum, cervix, colon, duodenum, endometrium, fallopian tube, heart, ileum, kidney, liver, lung, lymph node, nerve, oesophagus, ovary, pancreas, parathyroid gland, parotid gland, pituitary gland, placenta, prostate gland, skin, spinal cord, spleen, stomach body, skeletal muscle, testis, thyroid gland, tonsil and ureter.

6.1 Preparation of tissues for ICC

Human tissues were obtained mostly from post-mortem samples. Tissues were cut into 5mm^3 chunks and mounted onto cork pieces using a drop of OCT compound (Tissue-Tek, Miles Inc, Elkhart,

USA). To freeze the tissues, 20 mls of isopentane was cooled in a bath of liquid nitrogen and the mounted tissues immersed for 30 seconds. The frozen tissues were then placed into a cryotube and immersed in liquid nitrogen for a further 30 seconds. Tissue blocks were stored frozen at -70°C.

To cut sections, OCT compound was applied to a cryostat chuck and the frozen tissue embedded. The chuck and tissue were then snap frozen for 30 seconds in liquid nitrogen. The tissue was then mounted onto a cryostat and 5 micron cryosections of each human tissue cut onto microscope slides.

6.2 Preparation of phage antibodies for ICC

Phage antibody clones were inoculated into 5ml 2TYAG in 50ml Falcon tubes and grown at 37°C with aeration for 5 hours. M13K07 helper phage was added to each tube at an moi of 10 and incubated stationary for 30 min at 37°C followed by gentle shaking for 30 min at the same temperature. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant removed. Cell pellets were resuspended in 5ml 2TYKA and incubated at 30°C overnight. Tubes were centrifuged at 3000 rpm for 10 min and the phage antibody supernatant collected from each tube and preblocked with 1% BSA before use in ICC.

6.3 ICC on sections of normal human tissue

Human tissue sections were fixed by immersion in acetone at ambient temperature for 10 min, air dried and then washed once for 10 min in PBS. Sections were blocked in 5 µg/ml streptavidin in PBS for 15 min, washed 3 times in PBS and incubated in 10 µg/ml biotin in PBS for 15 min. Sections were washed 3 times in PBS and then incubated for 30 min in PBS containing 1% BSA (fatty acid free). Phage antibody supernatants preblocked in 1% BSA were incubated on the sections for 2 hr at ambient temperature. Slides were washed 3 times in PBST and incubated with an anti-M13-HRP conjugate

(Pharmacia) diluted 1/500 in PBS containing BSA. Sections were washed 3 times in PBST and a biotin tyramine amplification step then carried out. Biotin tyramine amplification consisted of incubation of the section with biotin tyramine diluted 1/600 in 50mM Tris-HCl, pH 7.4 containing 0.03% hydrogen peroxide for 10 min at room temperature, after which the slides was washed in 3 times in PBST. Sections were then incubated for 30 min in streptavidin-HRP complex (DAKO KO377) diluted in PBS, and then washed 3 times in PBST and once in PBS. Sections were stained by incubation with 3-amino-9-ethyl-carbazole (AEC, Sigma). AEC substrate was prepared by diluting a stock solution (2.4 mg/ml of AEC dissolved in dimethylformamide) 1:10 in 20mM sodium acetate buffer, pH 5.2 and adding 0.15% (v/v) of H_2O_2. 100ml of substrate solution was then pipetted onto each section and incubated for 5-10 min followed by washing in water to stop colour development. The slides were then counterstained with haemotoxylin (DAKO) for <5 seconds and then washed 3 times in water. Washed sections were then coated in aqueous mount and a glass cover slip applied.

6.3.1 Adipocyte Staining

108 antibodies, which had been isolated by virtue of their binding to adipocyte plasma membranes in ELISA, were first screened by phage antibody ICC on adipose tissue sections. 85 of the antibodies stained adipocytes on adipose tissue sections whereas 26 did not. This is either a consequence of the insensitivity of the ICC technique relative to ELISA, or that the antibodies recognise an epitope exposed on adipocyte plasma membranes but not on native adipocytes. All the clones that recognised adipocytes in adipose sections were subsequently shown to stain adipocytes in any tissue that contains adipocytes, for example, breast, skeletal muscle, skin, peripheral nerve, heart, thyroid gland, adrenal gland and parotid gland. This was also true in ELISA using

different adipocyte plasma membrane preparations (see 5.4).

Qualitatively, therefore, all the 85 different antibodies appear to recognise all fat depots. Quantitatively, adipocyte membrane staining fell into 3 categories:

1) strong uniform staining
2) "weak patchy" - characterised by antibody binding to the cytoplasm surrounding the adipocyte nucleus (which is flattened to the side of the cell),
3) "dotted patchy" - characterised by an incomplete but regular staining of the membrane

This variation in adipocyte staining pattern is most likely due to the density of the target antigen and/or to a lesser extent the affinity of each antibody for its antigen.

6.3.2 Cross-reactivity with Human Tissues

In addition to adipocyte staining, all antibodies also recognised at least one other cell type present in one or more human tissues.

6.3.2.1 Common cross-reactivity:

ICC patterns emerged whereby certain tissues were frequently recognised by many of the anti-adipocyte antibodies. The 9 tissues, apart from adipose, most frequently bound were spleen, heart, kidney, colon, liver, skin, striated muscle, tonsil and testis (see Table 6).

A description of the cell types and structures recognised by the anti-adipocyte antibodies in these tissues is summarised below, each exemplified by one or more antibodies:

(a) Spleen

red pulp of spleen, primarily to the sinusoidal network

(b) Heart and Striated muscle
capillaries that line either the cardiac or striated muscle fibres, walls of larger blood vessels

(c) Kidney
capillaries, larger blood vessels, general punctate staining throughout the kidney, kidney tubules and glomeruli

(d) Colon
capillaries, larger blood vessels, smooth muscle in the muscularis externa

(e) Liver
general punctate staining of hepatocytes, liver sinusoids

(f) Skin
capillaries, larger blood vessels, smooth muscle, sebaceous glands

(g) Tonsil:
post-capillary venules, blood vessels in the germinal centres, general punctate staining throughout the tonsil, lymphocytes

(h) Testis
capillaries, larger blood vessels, seminiferous tubules

6.3.2.2 Infrequent cross-reactivity

For a smaller proportion of the antibodies, binding to tissues other than those described in 6.3.2.1 was observed. The tissues and cell types recognised are described below and exemplified by one or more antibody:

(a) Breast - mammary lobules (Fat 2, Fat 7, Fat 19 and Fat 20)

(b) Thyroid gland - large blood vessels (Fat 1, Fat 26, Fat 36, Fat 40 and Fat 97)

(c) Peripheral nerve - epineurium tissue (Fat 31)

(d) Cerebellum - granular layer (Fat 17)
- molecular layer (Fat 29)

- connective tissue (Fat 117)

(e) Endometrium - epithelial cells (Fat 36)

(f) Myometrium - blood vessels (Fat 115)

(g) Lung - connective tissue and large blood vessels (Fat 40)

6.3.2.3 Extensive ICC Screen

A number of the antibodies have been extensively screened on a large panel of normal human tissues. These screens allow us to build a complete tissue binding profile for individual antibodies. For example, the antibody Fat 13 has been screened on 37 normal male and female tissues: adrenal gland, aorta, bladder, blood vessels, bone marrow, breast, cerebrum, cerebellum, cervix, colon, duodenum, endometrium, fallopian tube, heart, ileum, kidney, liver, lung, lymph node, nerve, oesophagus, ovary, pancreas, parathyroid gland, parotid gland, pituitary gland, placenta, prostate gland, skin, spinal cord, spleen, stomach body, skeletal muscle, testis, thyroid gland, tonsil and ureter. Fat 13 binding was restricted to adipocytes and to capillary endothelial cells in the following tissues:

(a) Adipocytes in breast, colon, skin, heart, striated muscle, nerve, parotid gland, adrenal gland and parathyroid gland

(b) Capillary endothelial cells in heart, colon, striated muscle, breast, cerebellum skin and liver (sinusoids).

Thus, the phage antibody Fat 13 recognises an epitope/antigen that is present on the adipocyte cell surface that is shared only with capillary endothelial cells. This antibody therefore shows a remarkable specificity and identifies an antigenic link between adipocyte and vasculature biology.

6.4 Summary

A method has been successfully developed to allow analysis of phage antibodies by ICC. Of 108 anti-adipocyte antibodies

identified by ELISA, 85 were shown to bind to native adipocytes in situ. These 85 clones have been screened by ICC on a panel of normal human tissues.

All the antibodies showed cross-reaction with one or more of the tissues screened. This cross-reaction was often limited to only a few cells in a particular tissue, for example to capillary endothelial cells in colon. As the antibodies were all 'adipocyte specific' in ELISA (see Example 5), the ability of ICC to visualise antibody binding to relatively minor tissue components demonstrates its use as a tool in the assessment of antibody binding profiles.

One feature of the ICC profiles of the anti-adipocyte antibodies was the cross-reaction observed in a number of common tissues. The most frequently bound tissues were spleen, heart and colon and to a lesser extent skeletal muscle and kidney. Other than adipocytes, the most commonly bound cells/structures observed in these tissues were capillary endothelial cells and the smooth muscle walls of large blood vessels.

The ICC profiles raise interesting implications for the relationship between adipocytes and other cell types. For example, it is well established that adipocytes and muscle cells both differentiate from the same precursor, or stem cell. That some of the antibodies described here bind to both adipocytes and smooth muscle suggests that the two cell types continue to express common antigens post-differentiation of the stem cell. Thus the antibody panel may be used to study development and biology of the precursor cell through to the mature adipocyte.

Moreover, the common staining patterns that were observed provide indication that there are antigens which are shared

by adipocytes and other cell types and that these in turn reflect a common biology or pathology. For example, the binding of some antibodies (e.g. Fat 13) to adipocytes and blood vessels indicates a common antigen in these two tissues. This antigen may therefore be involved in both adipocyte and blood vessel biology and pathological states where the two cell types are involved, for example in atherosclerosis. Alternatively, the cardiovascular staining pattern link between adipocytes and heart tissue may be connected to the high incidence of heart disease in obese individuals. Hence, the antibody panel is useful in further characterisation of the antigens on the adipocyte cell surface, their biology and the link with a variety of pathological conditions.

EXAMPLE 7: CHARACTERISATION OF ANTIGENS RECOGNISED BY THE ANTI-ADIPOCYTE ANTIBODIES

The panel of anti-adipocyte antibodies were isolated by (a) panning on either the adipocyte cell surface or on intact adipose tissue, or by (b) ProxiMol using a rabbit polyclonal antiserum that had been raised against human adipocyte plasma membranes. As a consequence of these selections, an extensive panel of antibodies that bind to the cell surface of adipocytes has been generated and these may be used to recognise a variety of different epitopes/proteins which may be important to adipocyte biology.

Experiments were performed to characterise the antigens bound by some of these anti-adipocyte antibodies, allowing for their identification.

7.1 Characterisation of Antigens using Protein Chemistry

Four antibodies were investigated in the first instance: Fat 13, Fat 37, Fat 40 and Fat 41. Of these antibodies, Fat 13, 37 and 41 were screened as rat chimeric IgG molecules (i.e.

IgG composed of rat constant domains coupled to human variable domains) whereas Fat 40 was screened as a scFv which had been purified by IMAC. All antibodies bound to adipocyte plasma membranes in ELISA and did not cross-react with the other cell types tested (see example 5 for details). In ICC, all clones strongly stained adipocytes in the human tissues screened. In addition, Fat 13, 37 and 41 all bound to capillaries in a number of tissues (particularly in heart). However these antibodies had different ICC tissue profiles, for example Fat 13 staining was restricted to adipocytes and capillaries whereas Fat 37 was also shown to bind the basal lamina in kidney. The ICC profile of Fat 40 was characterised by adipocyte and smooth muscle staining in many tissues.

7.2 SDS-PAGE western blotting

7.2.1 Method

Adipocyte plasma membranes, prepared as described in example 1, were boiled in SDS-PAGE sample buffer for 5 minutes at 90°C (2x SDS-PAGE sample buffer: 0.5M Tris-HCl pH6.8, 20% (v/v) glycerol, 10% (w/v) SDS, 20mM DTT, 0.1% (w/v) bromophenol blue). The membranes were then loaded, approximately 10µg per lane, onto 14% polyacrylamide gels supplied by Novex (Cat no. EC64855), in addition to a full range (250 to 10 kDa) of molecular weight marker. Samples were electrophoresed in SDS-PAGE running buffer (25mM Tris, 192mM glycine, 0.1% (w/v) SDS) for 2-3 hours at 100V and maximum current. Electrophoresis was stopped when the bromophenol blue dye front had reached the bottom of the gel. Proteins were then transferred from the gel to PVDF membrane. Blotting cassettes were prepared by soaking the following in transfer buffer (12mM Tris, 96mM Glycine, 20% (v/v) methanol) and then assembling in order:

(a) Three scotchbrite pads

(b) One piece of 30mm thick blotting paper

(c) Gel
(d) PVDF membrane
(e) One piece of 30mm thick blotting paper
(f) Three scotchbrite pads

The assembled cassette was inserted into the Novex blotting tank and immersed in transfer buffer, with the PVDF nearest the anode. Transfer of proteins from gel to PVDF was achieved overnight at 10 volts, maximum current and 4°C. PVDF membranes were then removed to a separate dish and incubated in 3% Marvel in PBS (Blotto) for 1 hour with shaking. The membranes were washed twice in PBS and then incubated with the primary antibodies at 10µg/ml in Blotto for 1 hour.

For scFv western blots, the PVDF membrane was washed three times in PBST and then incubated with the anti-myc antibody 9E10 diluted 1:100 in Blotto for 1 hour. After a further three washes in PBST, scFv westerns were then incubated with an anti-mouse IgG HRP conjugate antibody (Sigma) diluted 1:1000 in Blotto for 1 hour.

Rat IgG westerns were washed three times in PBST and then incubated with an anti-rat Ig Kappa light chain HRP conjugate antibody (Pharmingen) diluted 1:3000 in Blotto for 1 hour.

For both systems, blots were extensively washed in PBST (3 washes of 15 minutes) and then once in PBS for 15 minutes. Blots were then developed using the ECL detection system (Amersham). The blots were incubated with the substrate luminol which emits light when oxidised by HRP. This light emittance was then detected on photosensitive film.

7.2.2 Result of SDS-PAGE Western Blotting

Of the antibodies screened on adipocyte plasma membranes, only fat 37 has been shown to bind to its target antigen, a

protein of approximately 35KDa. The lack of binding seen for the other clones tested suggests that they recognise conformationally dependant epitopes. All proteins are denatured under SDS-PAGE conditions and it is unlikely that conformational epitopes would remain intact, resulting in an apparent loss of antibody reactivity.

7.3 Native-PAGE western blotting

7.3.1 Method

To avoid denaturation of target antigens, adipocyte plasma membranes were electrophoresed under near native conditions. As most membrane proteins require the presence of detergents to maintain solubility in aqueous solution, the SDS was replaced with a milder ionic detergent, deoxycholic acid. This detergent is similar to SDS in that it also carries an overall negative charge, thus all dissolved proteins will migrate toward the anode during electrophoresis. To confirm that this detergent is non-denaturing, all the antibodies were screened by ELISA against adipocyte plasma membranes solubilised in deoxycholic acid. No loss of binding was observed for any antibody (Fat 13, 37, 41 or 10C12).

Therefore, in this procedure adipocyte plasma membranes were not boiled but were loaded directly onto 4% Novex gels (Cat no. EC60555) in native loading buffer (2x: 0.5M Tris-HCl pH 8.8, 20% (v/v) glycerol, 0.1% (w/v) bromophenol blue). Samples were electrophoresed in native running buffer (25 mM Tris, 192 mM glycine, 2 mM deoxycholic acid) for 2-3 hours at 100V and maximum current. Electrophoresis was stopped when the bromophenol blue dye front had reached the bottom of the gel. The proteins were then transferred from the gel to PVDF membrane in native transfer buffer (12 mM Tris, 96 mM Glycine, 0.2 mM deoxycholic acid). The remainder of the blotting procedure followed that described in 7.2.1.

7.3.2 Result of Native Western Blotting

On native Western blots, all of the antibodies except Fat 13 were demonstrated to bind to their target antigen. However, the molecular weights of the antigens cannot be deduced from these native gels, as proteins do not separate according to size but to overall net charge. Under these conditions it was apparent that Fat 41 was binding to a protein with the same overall net charge as Fat 37. Coupled with the SDS-PAGE data, this result suggests that these two antibodies are binding to different epitopes of the same 35 kDa protein. Fat 40 also bound to a protein but to one with a different net charge to that bound by Fat 37 and Fat 41 - a result which suggests that Fat 40 is binding to a different antigen. The lack of binding seen for Fat 13 could be a consequence of antibody binding to a conformational epitope which is also sensitive to deoxycholic acid as well as SDS. Both detergents are ionic but deoxycholic acid is considerably milder and would result in less disruption to the overall conformation of the protein. This enabled the antigens for Fat 40 and 41 to be recognised but for Fat 13 an alternative detergent to deoxycholic acid, e.g. Triton, would have to be considered for further western blots.

7.4 Effect of temperature on Antigen Stability

7.4.1 Method

This study was performed as a follow up to the Western blots to gain further information on the stability of the antigens recognised by the antibodies. Adipocyte plasma membranes, diluted to 10µg/ml in PBS, were heated in 5°C increments from 50°C to 75°C for 20 minutes. The heated plasma membranes were then cooled to room temperature and coated directly onto Falcon 96 well polystyrene plates overnight at 4°C.

An ELISA was then performed with each antibody, i.e. Fat 13, Fat 37 & Fat 41 rat chimeric IgG each at 1µg/ml and Fat 40

scFv at 10mg/ml. Initially, the 96 well plates were washed once with PBS and 200µl of 3% Marvel in PBS (Blotto) added to each well for 1 hour at 37°C. The plates were washed twice in PBS and then incubated with the primary antibodies diluted in Blotto for 1 hour at 37°C.

For a scFv ELISA, plates were washed three times in PBST followed by three washes in PBS and then incubated with the anti-myc antibody 9E10 diluted 1:250 in Blotto for 1 hour at 37°C.

Rat IgG ELISAs were washed three times in PBST followed by three washes in PBS and then incubated with an anti-rat IgG_{2b} antibody (Pharmingen) diluted 1:1000 in Blotto for 1 hour at 37°C.

For both systems, a further three washes in PBST and PBS were performed and the plates probed with an anti-mouse IgG HRP conjugate antibody (Sigma) diluted 1:1000 in Blotto for 1 hour at 37°C. The plates were washed three times in PBST followed by three washes in PBS and then developed using TMB substrate until a sufficient level of colour had developed. Developing was stopped by the addition of ½ volume of 0.5M H_2SO_4 and the absorbances measured at 450 nm.

7.4.2 Result of Antigen Stability Experiment

From Figure 2 it can be seen that Fat 37 and Fat 41 follow similar profiles, i.e. their epitopes are not affected by heating to 55°C but are both denatured at 65°C. This result supports the observations seen on the Western blots in that the two antibodies appear to be binding to the same antigen. Fat 13 also shows a similar profile to that seen for Fat 37 and Fat 41. However, Fat 40 gives a distinctly different profile to the other antibodies suggesting, again, that it is binding to a different antigen.

7.5 Determination of pI using Rotofor

The Rotorfor system (Biorad) fractionates complex protein samples in free solution using preparative IEF. The system uses ampholytes to generate linear pH gradients which are then used to fractionate proteins on the basis of their pI (isoelectric point).

This Rotorfor system was used to fractionate proteins from an adipocyte plasma membrane preparation. In total, 1mg of human adipocyte plasma membranes solubilised in 0.1% (v/v) Triton X-100 were applied to the Rotorfor cell. These were mixed with an ampholyte buffer ranging from pH 3 to pH 10 and focussed overnight (10W, 3000V max and 150mA max). Using a vacuum assisted system, the fractions were harvested by simultaneous aspiration into 20 separate tubes. As the Rotorfor system is non-denaturing the resolved proteins can be coated directly onto 96 well polystyrene plates for ELISA analysis, avoiding the need to perform any western blotting. A 1:2 dilution of each of these fractions was coated onto Falcon 96 well plates and probed with Fat 13, Fat 37, Fat 40 and Fat 41. The ELISA methods employed for these antibodies were identical to those described in 7.4.1 above.

7.5.2 Evaluation of IEF Results

ELISA analysis of adipocyte plasma membrane proteins resolved by Rotorfor IEF revealed that Fat 13, Fat 37 and Fat 41 were all producing similar profiles of binding. For each of these antibodies the pI of the antigen was resolved over a wide pH range, from pH 5.5 to 8. Although the antigen/s bound by these antibodies did not focus particularly well at first, a more accurate pI was obtained by performing a second Rotofor run using a narrower pH gradient, i.e. pH 5 to 8. The pI of the antigen recognised by Fat 37 was determined to be between pH 5.5 to 6. The antigen bound by Fat 40 focussed to a narrow pH range in the first run and the pI was determined as pH 6.

7.6 Summary

We have performed a preliminary characterisation of the antigens bound by four anti-adipocyte antibodies: Fat 13, Fat 37, Fat 40 and Fat 41. One benefit of such a study would be the identification of important molecular characteristics for each antigen, for example molecular weight and isoelectric point. This information could then be directly applied to facilitate antigen purification and identification from the target tissue.

On SDS-PAGE westerns, only Fat 37 bound to its (35KDa) antigen. The other antibodies did not bind on these blots presumably because their epitopes are conformationally dependent. However, Fat 37, Fat 40 and Fat 41, but not Fat 13, were shown to bind their respective antigens on native westerns. On these native westerns it was apparent that Fat 37 and Fat 41 were binding to the same antigen whereas Fat 40 was binding to a different antigen.

A similar conclusion was drawn from the antigen stability study. In this study, the loss in binding of Fat 37 and Fat 41 to heat denatured adipocyte plasma membranes was observed at identical rates. Interestingly, Fat 13 also gave similar profiles to Fat 37 and Fat 41 in this study. However, Fat 40, again, followed a different profile to the other antibodies.

The isoelectric points for each antigen were determined by IEF analysis. The pI was approximately the same for all the antigens, i.e. in the pH range 5.5 to 8. For the antigens recognised by Fat 37 and Fat 40, the pI was resolved further to pH 5.5 to 6 and pH 6, respectively.

In conclusion, these characterisation studies provide indication that Fat 37 and Fat 41 are binding to the same antigen and that Fat 40 is binding to a different antigen.

This conclusion is also supported by the ICC profiles observed for these antibodies: Fat 37 and Fat 41 have been shown to bind adipocytes and capillaries in many tissues whereas Fat 40 binds to adipocytes and smooth muscle. The characterisation studies did not yield sufficient data on the antigen bound by Fat 13 to make any firm conclusions. However, Fat 13 has a similar ICC profile to Fat 37 and 41 and also closely follows the profiles of these antibodies in the antigen stability study. Thus it may be the case that Fat 13 is also binding to the same antigen as Fat 37 and Fat 41.

7.7 Antigen Identification

In order to determine the antigens bound by any of the anti-adipocyte antibodies a number of techniques could be employed. These are detailed in the following section.

7.7.1 Protein purification

7.7.1.1 Detergent solublisation

Membrane proteins are generally not directly soluble in aqueous solutions. In order to make the proteins soluble they must first be treated with detergents. Which detergent is useful for which protein is determined empirically. The detergent is added to a membrane preparation from human adipocytes at the CMC. The CMC is the concentration of detergent above which the detergent forms micelles in aqueous solution. The membranes and detergent are then mixed extensively, if the protein can be solublised by the detergent it will be extracted from the membranes into the detergent micelles. The proteins remaining in the membrane can be precipitated by ultra centrifugation leaving the detergent solublised proteins in solution. The protein can be detected by ELISA. It is unlikely that a single protein will be solublised and the detergent extraction method will solublise many proteins. Further purification would then be necessary.

7.7.1.2 Immunoprecipitation

The detergent solublised protein is mixed with a critical concentration of antibody, allowing the formation of an antibody-antigen complex. This complex can be recovered in a number of ways, such as centrifugation, or by capture of the antibody-antigen complex on a protein a column, or if scFv is used on an anti-tag column. It may also be possible to produce affinity columns using the antibody and capturing the antigen directly onto the column.

7.7.1.3 Electrophoresis

Proteins can be separated on the basis of their charge and size. By treating the protein with a charged detergent such as the anionic detergent SDS the protein can be made to take on charge that is independent of the charged amino acids that the protein may contain. The amount of detergent taken up by the protein is dependent on the size of the protein and separating the proteins on the basis of charge will then be directly proportional to the size of the protein. When these proteins are placed in an electric field they will migrate towards the electrode carrying the charge opposite to the overall charge carried by the protein. This is achieved by forming a matrix onto which the proteins can be loaded and through which they migrate in the electric field (Laemmli 1970, Nature Vol 227 : 680–685). Following electrophoresis they remain in the matrix where they can be visualised by direct staining, western blotting, or can be eluted for further analysis to provide information on size and composition.

7.7.1.4 Amino acid sequencing

Once the antigen of interest has been purified the sequence of amino acids that makes up the protein can be determined using Edman degredation. A chemical reaction is performed on the protein which labels the terminal amino acid at the

N-terminal end of the protein and separates it from the remainder of the protein. The amino acid separated from the protein can be identified and the process repeated. Many cycles of this are performed and the primary amino acid sequence of the protein identified. [G Allen in "Sequencing of proteins and peptides" (TS Work and RH Burdon, Eds.) Elsevier, Amsterdam, New York 1981].

7.7.1.5 Directed antigen biotinylation

The size of unknown antigens can also be determined by direct biotinylation of the antigen using biotin tyramine reagent. Solubilised membranes containing the antigen are incubated with HRP conjugated antibody or antibody fragment that binds the antigen. Biotin tyramine and hydrogen peroxide are then added and incubation carried out at room temp for 10 mins. An aliquot of the reactions is mixed with the appropriate volume of SDS loading buffer and heated to 95°C for 5 min. The proteins are loaded onto a 14% acrylamide SDS gel and separated by electrophoresis. The proteins are then transferred to a PVDF membrane and probed with streptavidin HRP. This will bind to the proteins labelled with the biotin in the biotin tyramine reaction. Proteins can be visualised by incubation with ECL reagents (Amersham) and exposure to either photographic emulsion or the image captured on a video camera system.

If specific labelling has occurred then the biotinylated proteins can be captured on a streptavidin surface of a SELDI mass spectrometer chip and the molecular weights determined. Treatment of the captured proteins with trypsin or other sequence specific protease would allow peptide mass mapping to be carried out and identification of the protein through comparison to mass maps from other known proteins.

7.7.2 Cloning

7.7.2.1 cDNA library construction

The mRNA from the cell type or tissue of interest (known to co-express antigen) is converted to a DNA copy using the enzyme reverse transcriptase. This converts the mRNA strand into a complementary DNA (cDNA) copy annealed to the mRNA. The RNA is then converted into DNA using a combination of the enzyme RnaseH, which introduces breaks in the RNA and DNApolimeraseI from E.coli which recognises the breaks and removes the RNA and replaces it with DNA. The cDNA is then ligated into a plasmid vector such that upstream of the cloned cDNA is a eukaryotic promoter and downstream is a transcriptional terminator. The vector should also carry a selectable marker and an origin of replication for amplification in E. coli and an origin for replication and maintenance in eukaryotic cells such as the SV40 origin (eg et al. 1994, PNAS USA 91, 9228-9232).

7.7.2.2 Expression screening

A library consisting of a number of clones in the region of 1 x 10^6 individuals is amplified in E. coli and the DNA purified. This is then used to transfect a eukaryotic cell line such as COS-7 cells (monkey kidney cells). The proteins encoded by the cDNA are expressed in the COS cells and transmembrane proteins would be present on the cell surface. Using antibodies to the protein of interest the cells expressing the antigen can be labelled and separated from the unlabelled cells either by cell sorting or antibody capture. The plasmids within them can be isolated and further rounds of transfection and selection performed if necessary. Once clonal plasmid encoding the antigen has been isolated the sequence of the cDNA can be determined and the amino acid sequence of the antigen deduced. An example of this type of approach was taken by Aruffo, A and Seed, B. 1987 Molecular cloning of a CD28 cDNA by a high efficiency COS cell expression system (Proc. Natl. Acad. Sci. (USA) 84: 8573).

7.7.3 Summary

A number of methods are described for identifying the antigens bound by the anti-adipocyte antibodies. A combination of these allows for identification of antigen and its cDNA and/or amino acid sequence. This sequence information can be analysed by searching protein databases for matches to known proteins. The results of these searches may lead to the identification of either novel adipocyte antigens or known antigens newly identified on the adipocyte cell surface.

All documents identified in this specification are incorporated by reference.

Table 1

Adipose Source	Selection on Adipocyte	Round	Input Titre	Output Titre
Abdominal subcutaneous	Cell surface	1	$1x10^{13}$	$1.25x10^{7}$
	Plasma membrane	2	$4x10^{11}$	$1.1x10^{6}$
	Plasma membrane	3	$5x10^{11}$	$2.25x10^{7}$
Abdominal subcutaneous	Cell surface	1	$4x10^{12}$	$3.6x10^{6}$
	Cell surface	2	$2x10^{12}$	$2x10^{8}$
	Cell surface	3	$1.2x10^{12}$	$7x10^{7}$
Omental	Cell surface	1	$4x10^{12}$	$1.3x10^{6}$
	Cell surface	2	$4x10^{12}$	$8x10^{7}$
	Cell surface	3	$1.5x10^{12}$	$2x10^{7}$
Lipoma	Cell surface	1	$1x10^{13}$	$1x10^{7}$
	Plasma membrane	2	$4x10^{11}$	$2x10^{6}$
	Plasma membrane	3	$5x10^{11}$	$1x10^{8}$

Table 2

Adipose Source	Temp.	Selection on Adipocyte	Round	Input Titre	Output Titre
Intact adipose	4°C	Tissue	1	$1x10^{13}$	$5x10^{8}$
		Plasma membrane	2	$1.95x10^{12}$	$1.9x10^{5}$
		Plasma membrane	3	$2.2x10^{13}$	$2.7x10^{8}$
Intact adipose	25°C	Tissue	1	$1x10^{13}$	$5x10^{8}$
		Plasma membrane	2	$2.8x10^{12}$	$5.4x10^{5}$
		Plasma Membrane	3	$1.2x10^{13}$	$2.2x10^{8}$

Table 3

Adipose Source	Temp.	Selection on Adipocyte	Round	Input Titre	Output Titre

Abdominal subcutaneous	4°C	Cell surface Cell surface	1 2	$1x10^{13}$ $1.5x10^{13}$	$3.5x10^{4}$ $2.6x10^{4}$
Abdominal subcutaneous	25°C	Cell surface Cell surface Plasma membranes	1 2 2	$1x10^{13}$ $2.2x10^{13}$ $1x10^{12}$	$1.5x10^{5}$ $8x10^{4}$ $2x10^{4}$

TABLE 4

Clone name	VH seg	VHCDR3	VL seg
FAT.1	VH3 DP49	NPRLAYDAFDI	VK1 DPK9
FAT.2	VH3 DP35	GGFEELFDGSFDI	VΛ3 DPL16
FAT.3	VH4 DP79	DRGFYGLDV	VΛ3 DPL16
FAT.4	VH3 DP53	DMWGTMDV	VΛ3 IGLV3S2
FAT.5	VH3 DP47	TIAYGDYGFDY	VΛ3 DPL 16
FAT.6	VH1 DP25	DIYYGSGYAFDI	VΛ3 DPL 16
FAT.7	VH3 DP47	SLYRWELLDF	VΛ3 DPL 16
FAT.8	VH3 DP49	DRRLQDAFDI	VK1 DPK9
FAT.9	VH3 DP49	ELGFSGPFDY	VK1 DPK9
FAT.10	VH1 DP25	FRGSGSFDV	VΛ3 DPL 16
FAT.11	VH3 DP47	DLGTGDSNYQFYYMDV	VΛ3 DPL 16
FAT.12	VH1 DP25	WGDFYYYMDV	VK1 DPK9
FAT.13	VH4 DP66	DNWGSLDY	VΛ6 IGLV6.S1
FAT.14	VH1 DP10	GWDT	VΛ3 DPL 16
FAT.15	VH4 DP79	YKWNTWFDP	VΛ3 DPL 16
FAT.16	VH3 DP47	SLYRWELFDF	VΛ3 DPL 16
FAT.17	VH3 DP47	SLFRWELFDL	VΛ3 DPL 16
FAT.18	VH4 DP71	DGESPLDFYFDF	VΛ3 DPL 16
FAT.19	VH3 DP49	DSWISGNFDY	VK1 DPK9
FAT.20	VH3 DP47	DYFDILTGPMDV	VΛ3 DPL 16
FAT.21	VH3 DP47	GGHYYGMDV	VΛ3 DPL 16
FAT.22	VH3 DP50	GWWSTNTYYFDY	VK1 DPK1
FAT.23	VH1 DP7	DSGYDGHGMDV	VΛ3 DPL 16
FAT.25	VH3 DP49	RWYGGSGYWGHFYSYMDG	VΛ3 DPL 16
FAT.26	VH3 DP46	YYISG	VΛ3 DPL 16
FAT.27	VH3 DP46	YYVSG	VΛ3 DPL 16
FAT.28	VH3 DP38	WGPPVYAKP	VΛ3 DPL 16
FAT.29	VH4 DP67	VNRYGSPBT	VΛ3 DPL 16
FAT.30	VH5 DP73	PHYPMTTDDAFDI	VΛ1 DPL 5/2
FAT.31	VH3 DP31	AAIASLGNCTSASCYNGAFDI	VΛ1 DPL 5/2
FAT.32	VH5 DP73	TDDGYNFAFDI	VΛ1 DPL 5/2
FAT.33	VH3 DP35	GSGDLDH	VΛ1 DPL 5/2
FAT.34	VH5 DP73	SMGTGWYVSYPDF	VΛ1 DPL 5/2
FAT.35	VH4 DP63	DTVGDYDSGGYYYSDS	VΛ1 DPL 5/2
FAT.36	VH1 DP14	DGVLDYYYGMDV	VΛ1 DPL 5/2
FAT.37	VH1 DP10	NYYYDSSGYYLYDAFDI	VΛ1 DPL 5/2
FAT.38	VH1 DP15	WPDCSGTSCYSPNY	VΛ1 DPL 5/2
FAT.39	VH1 DP14	YDARGYYYLDF	VΛ1 DPL 5/2
FAT.40	VH4 DP70	GYNWHYDDAFDI	VΛ3 DPL 16
FAT.41	VH1 DP14	EASLNLWPDPTWAFDI	VΛ1 DPL 5/2
FAT.42	VH1 DP14	GRAAADKTDY	VΛ1 DPL 5/2
FAT.44	VH1 DP14	KGLDRTYYMDVWGQVES	VΛ1 DPL 5/2
FAT.45	VH3 DP58	GGSSPAGVADY	VΛ1 DPL 5/2
FAT.46	VH3 DP32	SMIEGAFDI	VΛ1 DPL 5/2
FAT.48	VH3 DP47	AYSSEDY	VΛ1 DPL 5/2
FAT.49	VH3 DP47	GLTVFGVVNALDV	VΛ1 DPL 5/2
FAT.50	VH2 DP26	ERDYRLDY	VΛ1 DPL 5/2

FAT.52	VH1 DP14	SLVPTNCDN	VA1 DPL 5/2
FAT.53	VH5 DP73	HDVGYCSSSNCARRPEYFQH	VA1 DPL 5/2
FAT.54	VH1 DP10	DASIPDDTWDY	VA1 DPL 5/2
FAT.55	VH3 DP77	GQRLYIDS	VA1 DPL 5/2
FAT.56	VH6 DP74	DGSLGLDALDI	VA1 DPL 5/2
FAT.57	VH1 DP10	GKYAGNSGRHGMDV	VA1 DPL 5/2
FAT.58	VH3 DP35	DRDSSGYHI	VA1 DPL 5/2
FAT.59	VH3 DP61	DVYGMDV	VA1 DPL 5/2
FAT.60	VH1 DP25	RSGDVDTDMITSDAVDI	VA1 DPL 5/2
FAT.61	VH1 DP10	DYYDNGATNFDY	VA1 DPL 5/2
FAT.62	VH3 DP35	GDGSDYYAMDY	VA1 DPL 5/2
FAT.63	VH3 DP49	DGTTRTTATDYMDV	VA1 DPL 5/2
FAT.64	VH1 DP15	PGGLGAARPFDY	VA2 DPL 10
FAT.65	VH3 DP35	DARWFDP	VA1 DPL 5/2
FAT.66	VH3 DP77	EGIVGDGMDV	VA1 DPL 5/2
FAT.67	VH1 DP14	CAGCSGGDDAFDI	VA1 DPL 5/2
FAT.68	VH3 DP47	CQSISH	VA1 DPL 5/2
FAT.69	VH5 DP73	LSGQLLMEDAFDI	VA1 DPL 5/2
FAT.71	VH1 DP5	GGTPVVHDDAFEI	VA1 DPL 5/2
FAT.72	VH5 DP73	AGVAGGASDL	VA1 DPL 5/2
FAT.73	VH5 DP73	HNMIARPYDPFDI	VA1 DPL 5/2
FAT.74	VH1 DP10	DGQGRGWGRDWYFDI	Vk1 DPK 7
FAT.75	VH3 DP47	DLISPYYYYGMDV	VA1 DPL 5/2
FAT.76	VH1 DP14	GGGIRGMDA	VA1 DPL 5/2
FAT.77	VH3 DP31	EQADGPRIAVAGTGYMDV	VA1 DPL 5/2
FAT.78	VH3 DP31	AGRGDY	VA1 DPL 5/2
FAT.79	VH3 DP31	DRRTLDYFDY	VA1 DPL 5/2
FAT.82	VH3 DP48	DLPQYYYDSSGYYYPEYFQH	VA1 DPL 5/2
FAT.84	VH3 DP49	GYGSSYGGTS	VA3 DPL 16
FAT.86	VH1 DP31	QYSGYDYWDYFDY	VA1 DPL 5/2
FAT.87	VH3 DP77	SKVGGGNDY	VA1 DPL 5/2
FAT.88	VH1 DP88	DYSSRRYSYFDY	VA1 DPL 5/2
FAT.89	VH4 DP70	DRDTGBYFFDD	VA1 DPL 5/2
FAT.90	VH3 DP47	DPYCGSASYTYHAFDL	VA1 DPL 5/2
FAT.91	VH3 DP31	DKEYSSSYYFDY	VA1 DPL 5/2
FAT.92	VH3 DP49	DVLIHQTYKWFDP	VA1 DPL 5/2
FAT.93	VH3 DP32	DRNQYYDSGGYPDSFDI	VA1 DPL 5/2
FAT.94	VH3 DP35	LGTETIDY	VA1 DPL 5/2
FAT.95	VH3 DP31	DLSAGGMDV	VA2 DPL 12
FAT.96	VH1 DP14	TGSLFDY	VA1 DPL 5/2
FAT.97	VH1 DP10	DPLGTTGAFDI	VA2 DPL 11
FAT.98	VH3 DP58	EADYYYGMDV	VA1 DPL 5/2
FAT.99	VH3 DP47	DGEGTTGAEGQ	VA1 DPL 5/2
FAT.101	VH3 DP47	AYGSEDY	VA1 DPL 5/2
FAT.102	VH3 DP54	DLNPGQGGTYYDAFDI	VA1 DPL 5/2
FAT.103	VH3 YAC9	PGDSSGGMGRDY	VA1 DPL 5/2
FAT.104	VH3 DP58	GSAYYDILTGSGDDAFDI	VA1 DPL 5/2
FAT.105	VH1 DP10	EVIFFSEGMDV	VA1 DPL 2
FAT.106	VH1 DP75	DIDDSGYQY	VA2 DPL 11

FAT.107	VH3 DP47	DTYSGYDEAPTN	VλI DPL 5/2
FAT.108	VH1 DP14	AFNLGDSDYELEGDAFDI	VλI DPL 5/2
FAT.109	VH5 DP31	DISNIVLAPAATTSHFDY	VλI DPL 5/2
FAT.110	VH1 DP14	QYDIMTAYHTHGMDV	VλI DPL 5/2
FAT.111	VH1 DP10	DSGYDSPFY	VλI DPL 5/2
FAT.112	VH3 DP35	DFDSGGNSAIFDI	VλI DPL 5/2
FAT.113	VH1 DP88	CAEFCSDSNCPLDP	VλI DPL 5/2
FAT.114	VH3 DP49	DIAEGVGYYYMNV	VλI DPL 5/2
FAT.115	VH3 DP47	RANYYYLDV	Vλ3 DPL 16
FAT.116	VH3 VH3-8	GGTQCSFGVCATGG	VλI DPL 5/2
FAT.117	VH1 DP14	GGFCLNPVCYHGG	VλI DPL 5/2
FAT.118	VH3 DP54	GGLPCPCAACCSGG	VλI DPL 5/2

Where ND = Not determined

VH seg/VL seg = Closest germline VH/VL segment

Table 5

Clone name	VH SEQ ID NO. (amino acid)	VL SEQ ID NO. (amino acid)
FAT.1	2	4
FAT.2	6	8
FAT.3	10	8
FAT.4	12	14
FAT.5	16	18
FAT.6	20	22
FAT.7	24	26
FAT.8	28	4
FAT.9	30	4
FAT.10	32	26
FAT.11	34	36
FAT.12	38	40
FAT.13	42	44
FAT.14	46	26
FAT.15	48	26
FAT.16	50	26
FAT.17	52	54
FAT.18	56	26
FAT.19	58	4
FAT.20	60	62
FAT.21	64	26
FAT.22	66	68
FAT.23	70	26
FAT.25	72	26
FAT.26	74	8
FAT.27	76	8
FAT.28	78	26
FAT.29	80	26
FAT.30	82	84
FAT.31	86	88
FAT.32	90	84
FAT.33	92	84
FAT.34	94	84
FAT.35	96	84
FAT.36	98	84
FAT.37	100	102
FAT.38	104	88
FAT.39	106	84
FAT.40	108	36
FAT.41	110	84
FAT.42	112	88
FAT.44	114	116
FAT.45	118	84
FAT.46	120	116
FAT.48	122	84
FAT.49	124	84
FAT.50	126	84
FAT.52	128	84

FAT.53	130	84
FAT.54	132	84
FAT.55	134	84
FAT.56	136	84
FAT.57	138	84
FAT.58	140	84
FAT.59	142	84
FAT.60	144	84
FAT.61	146	84
FAT.62	148	84
FAT.63	150	84
FAT.64	152	154
FAT.65	156	84
FAT.66	158	84
FAT.67	160	84
FAT.68	162	84
FAT.69	164	84
FAT.71	166	84
FAT.72	168	170
FAT.73	172	84
FAT.74	174	176
FAT.75	178	84
FAT.76	180	84
FAT.77	182	88
FAT.78	184	84
FAT.79	186	84
FAT.82	188	84
FAT.84	190	26
FAT.86	192	84
FAT.87	194	84
FAT.88	196	84
FAT.89	198	84
FAT.90	200	84
FAT.91	202	84
FAT.92	204	84
FAT.93	206	84
FAT.94	208	84
FAT.95	210	212
FAT.96	214	84
FAT.97	216	218
FAT.98	220	88
FAT.99	222	224
FAT.101	226	116
FAT.102	228	84
FAT.103	230	84
FAT.104	232	84
FAT.105	234	236
FAT.106	238	240
FAT.107	242	84
FAT.108	244	84
FAT.109	246	84

FAT.110	248	84
FAT.111	250	88
FAT.112	252	254
FAT.113	256	258
FAT.114	260	84
FAT.115	262	264
FAT.116	266	88
FAT.117	268	84
FAT.118	270	84

Table 6

Clone Number	Adipose	Spleen	Heart	Kidney	Colon	Lung	Skin	Striated Muscle	Tonsil	Testis
1	+	+	+	-	-	+	-	-	-	-
3	+	-	nd	+	+	+	+	-	+	+
4	+	-	nd	-	-	+	-	+	-	+
5	+	+	nd	-	-	-	-	-	-	-
6	+	-	nd	-	+	+	-	+	-	-
8	+	+	nd	nd	nd	nd	nd	nd	nd	nd
10	+	-	nd	-	-	+	-	-	-	-
12	+	-	nd	-	+	-	+	-	-	-
13	+	-	+	-	-	-	-	+	-	+
15	+	-	nd	+	-	-	+	-	-	+	-
16	+	-	nd	+	+	+	-	-	-	-
17	+	-	nd	+	-	+	+	-	+	+
19	+	+	nd	nd	nd	nd	nd	nd	nd	nd
20	+	+	nd	nd	nd	nd	nd	nd	nd	nd
22	+	-	nd	-	+	+	+	+	-	+
23	+	+	nd	-	+	+	-	-	-	-
24	+	-	nd	-	+	+	-	-	-	-
26	+	+	+	-	+	-	-	-	-	-
27	+	+	nd	-	+	-	-	-	-	-
29	+	-	nd	-	+	-	+	-	+	-
30	+	+	nd	+	nd	nd	nd	nd	nd	nd
31	+	+	nd	+	+	+	-	+	-	+
32	+	-	nd	+	+	-	+	-	+	-
34	+	-	nd	+	-	-	-	-	+	-
36	+	+	nd	+	+	-	+	-	-	-
37	+	-	+	-	+	+	-	+	-	+
38	+	-	nd	-	+	-	-	-	-	-
39	+	+	nd	-	+	+	-	+	-	+
40	+	+	+	+	+	+	+	-	+	+
41	+	+	nd	+	+	+	-	+	-	+
42	+	+	nd	-	+	+	-	-	+	-
44	+	+	nd	-	+	-	-	-	-	-
46	+	-	nd	-	+	-	-	+	-	-

Clone Number	Adipose	Spleen	Heart	Kidney	Colon	Lung	Skin	Striated Muscle	Tonsil	Testis
48	+	-	nd	-	-	-	+	-	-	-
52	+	-	nd	+	-	-	-	+	-	-
57	+	-	+	-	-	-	-	-	-	-
58	+	-	+	-	-	-	-	-	-	-
60	+	-	nd	-	+	-	+	+	-	-
61	+	-	+	-	-	-	-	-	-	-
63	+	-	nd	+	-	-	-	-	-	-
67	+	-	+	-	-	-	-	-	-	-
68	+	+	nd	+	-	-	-	-	-	-
71	+	-	nd	+	-	-	+	+	-	-
72	+	+	nd	-	+	-	-	+	-	-
73	+	+	+	-	-	-	-	-	-	-
75	+	-	+	-	-	-	-	-	-	-
76	+	-	+	-	-	-	-	-	-	-
77	+	+	nd	-	+	+	+	+	-	+
78	+	+	nd	-	-	-	-	-	-	-
79	+	+	nd	-	-	-	-	-	-	-
80	+	+	nd	-	-	-	+	-	-	-
82	+	-	+	-	-	-	-	-	-	-
84	+	+	nd	-	-	-	-	-	-	-
86	+	-	+	-	-	-	+	-	-	-
87	+	+	nd	-	-	-	+	-	-	-
89	+	-	+	-	-	-	-	-	-	-
90	+	+	nd	-	-	-	+	-	-	-
91	+	+	nd	-	-	-	-	-	-	-
92	+	-	+	-	-	-	-	-	-	-
93	+	+	nd	-	-	-	-	-	-	-
94	+	+	nd	-	-	-	+	-	-	-
96	+	-	+	+	-	-	-	-	-	-
97	+	-	+	-	-	-	-	+	-	-
98	+	+	nd	-	+	+	+	+	-	+
99	+	+	nd	+	-	-	-	+	-	-
101	+	+	nd	+	-	-	-	+	-	-

Clone Number	Adipose	Spleen	Heart	Kidney	Colon	Lung	Skin	Striated Muscle	Tonsil	Testis
102	+	-	+	+	-	-	-	+	-	-
103	+	-	+	+	-	-	+	+	-	+
104	+	-	+	+	+	-	-	+	-	-
106	+	-	+	-	-	-	+	+	-	-
107	+	-	+	+	-	-	-	-	-	-
108	+	-	+	+	-	-	-	+	-	-
109	+	+	+	+	-	-	-	+	-	-
110	+	-	nd	+	-	-	+	-	-	-
111	+	+	nd	+	+	-	+	+	-	-
113	+	+	nd	-	-	-	-	+	-	-
114	+	+	+	-	-	-	-	-	-	-
115	+	-	nd	+	+	-	+	-	-	-
116	+	+	nd	+	+	-	-	+	-	-
117	+	-	nd	+	-	-	-	-	-	-
118	+	-	nd	+	-	-	-	+	-	-

+ = positive staining, - = no staining observed and nd = not determined

ANTIBODY VH AND VL DOMAIN AMINO ACID SEQUENCES AND CODING NUCLEOTIDE SEQUENCES

The cDNA and amino acid sequences of 108 anti-adipocyte antibodies according to embodiments of the present invention are detailed in this section. For each antibody, heavy chain sequence information is detailed first followed by the light chain sequence information. In the heavy chain amino acid sequences the CDR3 is underlined. A number of the anti-adipocyte antibodies share light chains in common: the sequences for these are not duplicated but are cross-referenced to each other.

CLAIMS

1. A library or panel of at least 10 different specific binding members, the library or panel comprising specific binding members each able to bind whole adipocytes and each comprising an antibody VH variable domain, wherein each antibody VH variable domain comprises a VH CDR shown in Table 4 and optionally has an amino acid sequence selected from the group consisting of those with a SEQ ID NO. listed in Table 5.

2. A library or panel according to claim 1 comprising at least 100 different antibody VH CDR's or antibody VH variable domains.

3. A library or panel according to claim 2 comprising or consisting of all 108 different VH domains of which the amino acid SEQ ID NO.'s are listed in Table 5.

4. A library or panel according to claim 2 comprising or consisting of all 108 different VH CDR3's shown in Table 4.

5. A library or panel according to any one of claims 1 to 4 wherein each VH domain is paired with a VL domain.

6. A library or panel according to claim 5 wherein the VL domain is or VL domains in the library or panel are selected from the group consisting of those of which the amino acid sequence has a SEQ ID NO. listed in Table 5.

7. A library or panel according to claim 6 wherein one or more VL domains in the library or panel is or are selected from the group consisting of those with SEQ ID NO.'S 4, 8, 26, 84, 88 and 116.

8. A method of obtaining one or more specific binding members able to bind an adipocyte antigen, the method including bringing into contact a library or panel of specific binding members according to any one of claims 1 to 7 and said adipocyte antigen, and selecting one or more specific binding members of the library or panel able to bind said adipocyte antigen.

9. A method according to claim 8 wherein specific binding members in the library or panel are displayed on the surface of bacteriophage particles, each bacteriophage particle containing nucleic acid encoding the antibody VH variable domain displayed on its surface, and optionally also a displayed VL domain if present.

10. A method according to claim 9 wherein bacteriophage particles displaying a specific binding member able to bind said adipocyte antigen are selected, and nucleic acid is taken from such a selected bacteriophage particle.

11. A method according to claim 10 wherein said nucleic acid is used in subsequent production of a specific binding member or an antibody VH variable domain, and optionally an antibody VL variable domain, by expression from nucleic acid with the sequence of nucleic acid taken from a selected bacteriophage particle displaying a specific binding member able to bind said adipocyte antigen.

12. A method according to any one of claims 8 to 11 comprising provision of a selected specific binding member or antibody VH domain of a selected specific binding member in isolated form.

13. A method according to any one of claims 8 to 11 comprising provision of a plurality of selected specific

binding members or antibody VH domains of a selected specific binding members in isolated form.

14. A method according to claim 13 comprising provision of a mixture of selected specific binding members or antibody VH domains of selected specific binding members in isolated form.

15. A method according to any one of claims 12 to 15 wherein a selected specific binding member or an antibody VH variable domain of a selected specific binding member optionally with a VL domain, a plurality of said specific binding members or antibody VH variable domains optionally with VL domains, or a mixture of said specific binding members or antibody VH variable domains optionally with VL domains, in isolated form is formulated into a composition including at least one additional component.

16. A method according to any one of claims 8 to 15 wherein a selected specific binding member or VH domain of a selected specific binding member is provided in a fusion protein with additional amino acids.

17. A method according to claim 16 wherein said additional amino acids provide an antibody constant region.

18. A mixture of 10 different specific binding members each comprising an antibody VH variable domain, obtainable from a library according to any one of claims 1 to 6, wherein each antibody VH variable domain has an amino acid sequence selected from the group consisting of the VH domains of Fat3 (SEQ ID NO. 10), Fat13 (SEQ ID NO. 42), Fat17 (SEQ ID NO. 52), Fat31 (SEQ ID NO. 86), Fat37 (SEQ ID NO. 100), Fat40 (SEQ ID NO. 108), Fat86 (SEQ ID NO. 192), Fat97 (SEQ ID NO. 216), Fat103 (SEQ ID NO. 230) and Fat106 (SEQ ID NO. 238).

19. A mixture of 10 different specific binding members each comprising an antibody VH variable domain, obtainable from a library according to any one of claims 1 to 6, wherein each antibody VH variable domain has an amino acid sequence comprising a CDR3 selected from the group consisting of the VH domains of Fat3, Fat13, Fat17, Fat31, Fat37, Fat40, Fat86, Fat97, Fat103 and Fat106 (the CDR3 sequences being shown in Table 4).

20. A composition comprising a plurality of different antibody VH variable domains obtainable from a mixture according to claim 18 or claim 19.

21. A composition according to claim 20 comprising any one or more of the antibody VH variable domains of Fat3 (SEQ ID NO. 10), Fat13 (SEQ ID NO. 42), Fat17 (SEQ ID NO. 52), Fat31 (SEQ ID NO. 86), Fat37 (SEQ ID NO. 100), Fat40 (SEQ ID NO. 108), Fat86 (SEQ ID NO. 192), Fat97 (SEQ ID NO. 216), Fat103 (SEQ ID NO. 230) and Fat106 (SEQ ID NO. 238).

22. A composition according to claim 21 comprising either or both of the antibody VH variable domains of Fat13 (SEQ ID NO. 42) and Fat40 (SEQ ID NO. 108).

23. A composition according to any one of claims 18 to 22 wherein one or more of said antibody VH variable domains is in a fusion with additional amino acids.

24. A composition according to any one of claims 18 to 23 wherein one or more of said antibody VH variable domains is in association with an antibody VL variable domain.

25. An antibody VH variable domain obtainable from a library or panel according to claim 3 and having an amino acid sequence of which the SEQ ID NO. is shown in Table 5.

26. An antibody VH variable domain obtainable from a mixture according to claim 18.

27. An antibody VH variable domain obtainable from a mixture according to claim 19.

28. A specific binding member comprising an antibody VH variable domain according to claim 26 or claim 27 and an antibody VL variable domain.

29. Nucleic acid encoding an antibody VH variable domain according to claim 26 or claim 27.

30. Nucleic acid encoding a specific binding member according to claim 28.

31. A host cell transformed with such nucleic acid according to claim 29 or claim 30.

32. A method of producing an antibody VH variable domain or specific binding member, the method comprising culturing host cells according to claim 31 under conditions for production of said antibody VH variable domain or specific binding member.

33. A method according to claim 32 further comprising isolating and/or purifying said antibody VH variable domain or specific binding member.

34. A method according to claim 32 or claim 33 further comprising formulating the said antibody VH variable domain or specific binding member into a composition including at least one additional component.

35. A method of obtaining one or more antigen molecules, the method comprising bringing into contact material suspected of containing an antigen of interest and a specific binding member or plurality or mixture of specific binding members as claimed in a preceding claim, and selecting one or more antigen molecules bound by said specific binding member, plurality or mixture thereof.

36. A method according to claim 35 further comprising providing a selected antigen molecule in an isolated and/or purified form.

37. A method according to claim 36 further comprising formulating said selected antigen molecule into a composition including at least one additional component.

Figure 1

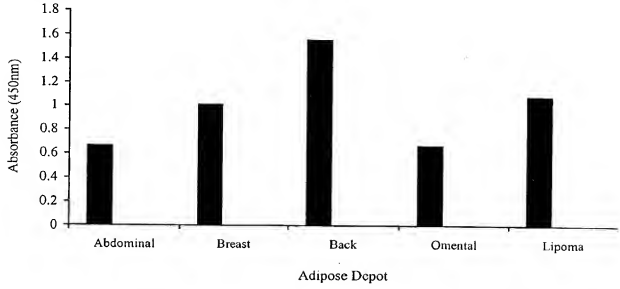
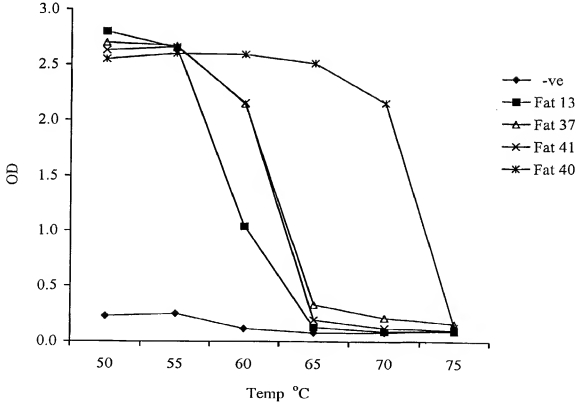


Figure 2



FAT 1 Heavy Chain DNA sequence SEQ ID NO. 1

CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTATTAAATACTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGCGAAACCCCAGACT
GGCCTATGATGCTTTTGATATCTGGGGCCAGGGGACAATGGTCACCGTCTCTTCA

FAT 1 Heavy Chain Amino Acid sequence SEQ ID NO. 2

Q V Q L V Q S G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V I S Y D G S I K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A R N P R L A Y D
A F D I W G Q G T M V T V S S

FAT 1 Light Chain DNA sequence SEQ ID NO. 3

GACATCGTGATGACCCAGTCTCTTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCA
TCACTTGCCGGGCCAGTCAGGGTATTAGTAGCTGGTTGGCCTGGTATCAGCAGAAACCAGG
GAGAGCCCCTAAGGTCTTGATCTATAAGGCATCTACTTTAGAAAGTGGGGTCCCATCAAGG
TTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG
ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCGGCCAAGGGAC
CAAGCTGGAGATCAAACGTGCGGCCGCACATCATCATCACCATCAC

FAT 1 Light Chain Amino Acid sequence SEQ ID NO. 4

D I V M T Q S L S T L S A S V G D R V T I
T C R A S Q G I S S W L A W Y Q Q K P G R
A P K V L I Y K A S T L E S G V P S R F S
G S G S G T D F T L T I S S L Q P E D F A

T Y Y C Q Q S Y S T P W T F G Q G T K L E
I K R A A A

FAT 2 Heavy Chain DNA sequence SEQ ID NO. 5

CAGGTGCAGCTGCAGGAGTCGGGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCTGAGACTCT
CCTGTACAGCCTCTGGATTCGCCTTCAGTGACCACTACATGAGTTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGATTTCATCCATTAGTACGAGTAGTATGTATATAAATTATGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTTCTGTGTTTTGGGGGGATTCGA
AGAATTATTTGATGGTTCTTTTGATATCTGGGGCCAAGGCACCCTGGTCACCGTCTCGAGT

FAT 2 Heavy Chain Amino Acid sequence SEQ ID NO. 6

Q V Q L Q E S G G R L G Q A W R V L R L S
C T A S G F A F S D H Y M S W V R Q A P G
K G L E W I S S I S T S S M Y I N Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y F C V L G G F E E L F
D G S F D I W G Q G T L V T V S S

FAT 2 Light Chain DNA sequence SEQ ID NO. 7

TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CTTGCCAAGGAGACAGTCTCAGAAGCTATTACACAAACTGGTTCCAGCAGAAGCCAGGACA
GGCCCCTCTACTTGTCGTCTATGCTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTGCGGCCGCACATCATCATCACCATCAC

FAT 2 Light Chain Amino Acid sequence SEQ ID NO. 8

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y T N W F Q Q K P G Q A
P L L V V Y A K N N R P S G I P D R F S G

S S S G N T A S L T I T G A Q A E D E A D
Y Y C N S R D S S G N H V V F G G G T K L
T V L G

FAT 3 Heavy Chain DNA sequence SEQ ID NO. 9

CAGGTACAGCTGCAGCAGTCAGGCCCAGGGCGGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CATGCTCTGTCTCTGGTGACTCCATCAGCAGTAGTAGTCACTACTGGAGCTGGATCCGCCA
GCCCCCAGGGAAGGGGCTGGAATGGATTGGCGATGTCAATCATGGTGGAAATACCAACTAC
AACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCC
TGAACCTGAAGTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGACCGGGG
TTTCTACGGTTTGGACGTCTGGGGCCAGGGCACCCTGGTCACCGTCTCGAGT

FAT 3 Heavy Chain Amino Acid sequence SEQ ID NO. 10

Q V Q L Q Q S G P G R V K P S E T L S L T
C S V S G D S I S S S S H Y W S W I R Q P
P G K G L E W I G D V N H G G N T N Y N P
S L K S R V T I S V D T S K N Q F S L N L
K S V T A A D T A V Y Y C A R D R G F Y G
L D V W G Q G T L V T V S S

FAT 3 Light Chain DNA and Amino Acid sequences

Identical to FAT 2 (SEQ ID NO.'s 7 and 8)

FAT 4 Heavy Chain DNA sequence SEQ ID NO. 11

CAGGTGCAGCTGCAGGAGTCCGGGGGAGGCTTGGTTCAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCCTCAGTCCCTATTGGATGCACTGGGTCCGCCAAGTTCC
AGGGAAGGGGCTGGAGTGGGTCTCACGTATAAATCCTGATGGGAGTAACACAGACTACGCG
GACTCCGTGAGGGGCCGATTCACCATGTCCAGAGACAACGCCAAGAACACGTTGTCTCTAG
AAATGAACAGTCTGAGAGCCGAGGACACGGCTGTATATTTTTGTGCAAGAGATATGTGGGG
GACCATGGACGTCTGGGGCCGAGGGACAATGGTCACCGTCTCGAGT

FAT 4 Heavy Chain Amino Acid sequence SEQ ID NO. 12

Q V Q L Q E S G G G L V Q P G G S L R L S
C A A S G F T L S P Y W M H W V R Q V P G
K G L E W V S R I N P D G S N T D Y A D S
V R G R F T M S R D N A K N T L S L E M N
S L R A E D T A V Y F C A R D M W G T M D
V W G R G T M V T V S S

FAT 4 Light Chain DNA sequence SEQ ID NO. 13

CTGCCTGTGCTGACTCAGCCCCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCACCATTA
CCTGTGGGGGAAACAAGATTGGAAGTAAAAGTGTGCATTGGTACCAGCAGCGGCCAGGCCA
GGCCCCTGTGGTGGTCATGTATTATGATAGTGTGACGATGCTTGAGCGATTCTCTGGCTCC
AATTCCGGAAACACGGCCTCCCTGACCATCACCAACGTCGAAGCCGGGGATGAGGCCGACT
ATTACTGTCAGGTGTGGCGTAGTGATACTGATCATGTGATATTCGGCGGAGGGACCAAGGT
CACCGTCCTAGGTGCGGCCGCACATCATCATCACCATCAC

FAT 4 Light Chain Amino Acid sequence SEQ ID NO. 14

L P V L T Q P P S V S V A P G K T A T I T
C G G N K I G S K S V H W Y Q Q R P G Q A
P V V V M Y Y D S V T M L E R F S G S N S
G N T A S L T I T N V E A G D E A D Y Y C
Q V W R S D T D H V I F G G G T K V T V L
G

FAT 5 Heavy Chain DNA sequence SEQ ID NO. 15

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGAAGTCCCTGAGACTCT
CTTGTGCAGGCTCTGGATTCACCTTTAGTAGCTATGCCATGAGTTGGGTCCGTCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGGTATTAGTGGTAGTGGTGGTAGCACATACTACACA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGACGACACGGCCGTATATTACTGTGCGAAAACGATCGCCTA
CGGTGACTATGGCTTTGACTACTGGGGCCGAGGAACCCTGGTCACCGTCTCCTCA

FAT 5 Heavy Chain Amino Acid sequence SEQ ID NO. 16

E V Q L V E S G G G L V Q P G K S L R L S
C A G S G F T F S S Y A M S W V R Q A P G
K G L E W V S G I S G S G G G S T Y Y T D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A D D T A V Y Y C A K T I A Y G D Y
G F D Y W G R G T L V T V S S

FAT 5 Light Chain DNA sequence SEQ ID NO. 17

TCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCCAAGG
GACCAAGCTGGAGATCAAACGTGCGGCCGCACATCATCATCACCATCAC

FAT 5 Light Chain Amino Acid sequence SEQ ID NO. 18

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N N R P S G I P D R F S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C N S R D S S G N H V V F G Q G T K L
E I K R

FAT 6 Heavy Chain DNA Sequence SEQ ID NO. 19

GAAGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCTTCAGTGACGGTCT
CCTGCAAGGCTTCTGGTTACACCTTTACCAGTTCTGGAATCAACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTCAGTGGATGGGGTGGATCAACGCTGCCAATGGTAAAACAACATACTCA
CAGAACTTCCAGGACAGACTCACCATTACCAGGGACGCCTCCGCGAGCACAGCCTACCTGG

AACTGAGCAGCCTGCGATCTGAAGACACGGCTGTGTATTACTGTGCGAGAGATATATACTATGGTTCGGG

FAT 6 Heavy Chain Amino Acid sequence SEQ ID NO. 20

E V Q L V Q S G A E V K K P G A S V T V S
C K A S G Y T F T S S G I N W V R Q A P G
Q G L Q W M G W I N A A N G K T T Y S Q N
F Q D R L T I T R D A S A S T A Y L E L S
S L R S E D T A V Y Y C A R D I Y Y G S G
Y A F D I W G R G T T V T V S S

FAT 6 Light Chain DNA sequence SEQ ID NO. 21

CAGTCTGTGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAATAAGCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTCATTCCCGGGACGGCAGTGGTAACCATGTGCTTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGT

FAT 6 Light Chain Amino Acid sequence SEQ ID NO. 22

Q S V L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N K R P S G I P D R F S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C H S R D G S G N H V L F G G G T K L
T V L G

FAT 7 Heavy Chain DNA Sequence SEQ ID NO. 23

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTATATCTGC

AAATGAACAGCCTGAGAGCCGAGGACACGGCCTTATATTACTGTGCGAAGTCTCTCTATCG
GTGGGAGCTTCTTGACTTCTGGGGCAAGGGGACAATGGTCACCGTCTCGAGT

FAT 7 Heavy Chain Amino Acid sequence SEQ ID NO. 24

Q V Q L V E S G G G L V Q P G G S L R L S
C A A S G F T F S S Y A M S W V R Q A P G
K G L E W V S A I S G S G G G S T Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A L Y Y C A K S L Y R W E L
L D F W G K G T M V T V S S

FAT 7 Light Chain DNA sequence SEQ ID NO. 25

TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTGCGGCCGCACATCATCATCACCATCAC

FAT 7 Light Chain Amino Acid sequence SEQ ID NO. 26

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N N R P S G I P D R F S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C N S R D S S G N H V V F G G G T K L
T V L G

FAT 8 Heavy Chain DNA Sequence SEQ ID NO. 27

CAGGTGCAGCTACAGCAGTGGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTATTAAATACTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCAGTTTATCTGC
AATTGACTGGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCAAGAGATCGGAGACT
ACAGGATGCTTTTGATATCTGGGGCCAAGGGACA

FAT 8 Heavy Chain Amino Acid sequence SEQ ID NO. 28

Q V Q L Q Q W G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V I S Y D G S I K Y Y A D S
V K G R F T I S R D N A K N S V Y L Q L T
G L R A E D T A V Y Y C A R D R R L Q D A
F D I W G Q G T

FAT 8 Light Chain DNA and Amino Acid sequence

Identical to FAT 1 (SEQ ID NO.'s 3 and 4)

FAT 9 Heavy Chain DNA Sequence SEQ ID NO. 29

GGGGTGCAGCTGGTGCAATCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTATTAAATACTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGCGAGAATTAGGATT
TAGTGGCCCCTTTGATTACTGGGGCCAGGGGACAATGGTCACCGTCTCGAGT

FAT 9 Heavy Chain Amino Acid sequence SEQ ID NO. 30

G V Q L V Q S G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V I S Y D G S I K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N

S L R A E D T A V Y Y C A R E L G F S G P
F D Y W G Q G T M V T V S S

FAT 9 Light Chain DNA and Amino Acid sequences

Identical to FAT 1 (SEQ ID NO.'s 3 and 4)

FAT 10 Heavy Chain DNA Sequence SEQ ID NO. 31

CAAGTACAGCTGCAGCAGTCAGGGGCTGAGGTGAAGAAGCTGGGCTCAGTGAAGGTTTCTG
CAAGGTTCTGGATACACTTCACTAGTCATGCTATGCATTGGGTGCGCCAGGCCCCCGGACA
AAGGCTTGAGTGGATGGGATGGATCAACGCTGGCAATGGTAAAATAAGATATTCACAGAGG
TTGCAGGGCAGAGTCACAATTACCAGGGATACATCCGCGAGCACAGCCTACATGGAGCTGA
GAAGCCTGAGATATGAAGACACGGCTGTCTATTACTGTGCGAGATTCCGTGGATCTGGAAG
TTTTGATGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

FAT 10 Heavy Chain Amino Acid sequence SEQ ID NO. 32

Q V Q L Q Q S G A E V K K L G S V K V S A
R F W I H F T S H A M H W V R Q A P G Q R
L E W M G W I N A G N G K I R Y S Q R L Q
G R V T I T R D T S A S T A Y M E L R S L
R Y E D T A V Y Y C A R F R G S G S F D V
W G Q G T L V T V S S

FAT 10 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 11 Heavy Chain DNA sequence SEQ ID NO. 33

CAGGTGCAGCTGGTGGAGTCTGGGGGAGTCGTGGTACATCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAGGGGACTGGAGTGGGTCTCAGGTCTTAGTGGTAGCGGTGGTAGTACATATTACGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAAAAGCCTGAGAGCCGAGGACACGGCCGTCTATTACTGCGCGAAAGATCTCGGCAC
CGGGGACAGCAACTATCAGTTCTACTACATGGACGTCTGGGGCCAAGGGACAATGGTCACC
GTA

FAT 11 Heavy Chain Amino Acid sequence SEQ ID NO. 34

Q	V	Q	L	V	E	S	G	G	V	V	V	H	P	G	R	S	L	R	L	S
C	A	A	S	G	F	T	F	D	D	Y	A	M	H	W	V	R	Q	A	P	G
R	G	L	E	W	V	S	G	L	S	G	S	G	G	S	T	Y	Y	A	D	S
V	K	G	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	K
S	L	R	A	E	D	T	A	V	Y	Y	C	A	K	D	L	G	T	G	D	S
N	Y	Q	F	Y	Y	M	D	V	W	G	Q	G	T	M	V	T	V	W	L	

FAT 11 Light Chain DNA Sequence SEQ ID NO. 35

TCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGGCAGCCCCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTCATTCCCGGGACAGCAGTGGTAACCATGTGCTTTTCGGCGGAGG
GACCAAGCTGACCGTCGTAGGT

FAT 11 Light Chain Amino Acid Sequence SEQ ID NO. 36

S	S	E	L	T	Q	D	P	A	V	S	V	A	L	G	Q	T	V	R	I	T
C	Q	G	G	S	P	R	S	Y	Y	A	S	W	Y	Q	Q	K	P	G	Q	A
P	V	L	V	I	Y	G	K	N	N	R	P	S	G	I	P	D	R	F	S	G
S	S	S	G	N	T	A	S	L	T	I	T	G	A	Q	A	E	D	E	A	D
Y	Y	C	H	S	R	D	S	S	G	N	H	V	L	F	G	G	G	T	K	L
T																				

FAT 12 Heavy Chain DNA Sequence SEQ ID NO. 37

GAGGTGCAGCTGGTGCAGTCTAGGGCTGCGGCGAGGAAGCCGAGGGCCTCAGTGCGGGTCT
CCTGCAAGGCTTCCGGTTACACCTTCACCAATAATGCTTTACATTGGGTGCGCCAGGCCCC
CGGACAAAGTCTTGAGTGGATGGGATGGATCAACACTGGCAATGGGATCACAAAATATTCA
CAGAGGTTTCGTGACAGAGTCACCATTACCAGGGACACATCCGCGAGCACAGTCTACATGG
AGGTGCACAGCCTGACACCCGGAGACACGGCTGTCTATTTCTGTGCGAGATGGGGAGACTT
CTACTACTACATGGACGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

FAT 12 Heavy Chain Amino Acid sequence SEQ ID NO. 38

E V Q L V Q S R A A A R K P R A S V R V S
C K A S G Y T F T N N A L H W V R Q A P G
Q S L E W M G W I N T G N G I T K Y S Q R
F R D R V T I T R D T S A S T V Y M E V H
S L T P G D T A V Y F C A R W G D F Y Y Y
M D V W G Q G T L V T V S S

FAT 12 Light Chain DNA Sequence SEQ ID NO. 39

GACATCGTGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCA
TCACTTGCCGGGCCAGTCAGGGTATTAGTAGCTGGTTGGCCTGGTATCAGCAGAAACCAGG
GAGAGCCCCTAAGGTCTTGATCTATAAGGCATCTACTTTAGAAAGTGGGGTCCCATCAAGG
TTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG
ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTAACCCCGTGGACGTTCGGCCAAGGAC
CAAGCAGGAGATCAAACGT

FAT 12 Light Chain Amino Acid Sequence SEQ ID NO. 40

D I V M T Q S P S T L S A S V G D R V T I
T C R A S Q G I S S W L A W Y Q Q K P G R
A P K V L I Y K A S T L E S G V P S R F S
G S G S G T D F T L T I S S L Q P E D F A
T Y Y C Q Q S Y S N P V D V R P R T K Q E
I K R

FAT 13 Heavy Chain DNA Sequence SEQ ID NO. 41

CAGGTGCAGCTACAGCAGTGGGGCTCAGGACTGATGAAGCCCTCGGAGACCCTGTCCCTCA
CCTGCACTGTCTCTGGTGACTCTATTAACAATAATGAATATCACTGGGCCTGGATCCGCCA
GCCCCCAGGGAAGGGACTGGAGTGGATTGGGTATATCAATCACAGAGGAATTGGAACCACC
AACCACAACCCCTCCCTCAAGAGTCGAGTCACCATGTCAGTAGACACGTCCAAGAATCAGT
TCTCCCTGAAGCTGAGCTCTGTGACCGCTGCGGACACGGCCGTATATTATTGTGCGAGAGA
TAACTGGGGATCGCTGGACTACTGGGGGCGGGGGACCACGGTTACCGTATCAAGT

FAT 13 Heavy Chain Amino Acid sequence SEQ ID NO. 42

Q V Q L Q Q W G S G L M K P S E T L S L T
C T V S G D S I N N N E Y H W A W I R Q P
P G K G L E W I G Y I N H R G I G T T N H
N P S L K S R V T M S V D T S K N Q F S L
K L S S V T A A D T A V Y Y C A R D N W G
S L D Y W G R G T T V T V S S

FAT 13 Light Chain DNA Sequence SEQ ID NO. 43

GCACTTAATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAA
CCATCTCCTGCACCCGCAGCAGCGGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCA
GCGCCCGGGCAGTGCCCCCACCACTGTGATCTATGAGGATAACCAAAGACCCTCTGGGGTC
CCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCCTCCCTCACCATCTCTG
GACTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGATAGCAGCAATCGGGT
GTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT

FAT 13 Light Chain Amino Acid Sequence SEQ ID NO. 44

A L N F M L T Q P H S V S E S P G K T V T
I S C T R S S G S I A S N Y V Q W Y Q Q R
P G S A P T T V I Y E D N Q R P S G V P D
R F S G S I D S S S N S A S L T I S G L K
T E D E A D Y Y C Q S Y D S S N R V F G G
G T K L T V L G

FAT 14 Heavy Chain DNA Sequence SEQ ID NO. 45

AAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCGTCGGTGAAGGTCA
CCTGCGAGGCTTCCGGAGGCTACTTCAGTAGTTATGCTTTCAACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGAATCATCCCTTTGTTTGGTACACCAAACTTCGCA
CAGAAGTTGCAAGGCAAGGTCACGATTACCGCGGACGAGTCCACGAGCACAATCTACCTGG
AGGTGAGCAACCTGACATCTGAAGACACGGCCGTCTATTTCTGTGCGAGAGGTTGGGACAC
CTGGGGCCAAGGCACCCTGGTCACCGTATCGTCCA

FAT 14 Heavy Chain Amino Acid sequence SEQ ID NO. 46

K V Q L V Q S G A E V K K P G S S V K V T
C E A S G G Y F S S Y A F N W V R Q A P G
Q G L E W M G G I I P L F G T P N F A Q K
L Q G K V T I T A D E S T S T I Y L E V S
N L T S E D T A V Y F C A R G W D T W G Q
G T L V T V S S

FAT 14 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 15 Heavy Chain DNA Sequence SEQ ID NO. 47

GAGGTGCAGCTACAGCAGTGGGGCCCAGGACTGCTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGTAGTGTTTCTCGTGACTCCGTGAACAGGAATAGTAACTACTGGGGCTGGATCCGCCA
GACCCCAGGGAAGAAGCTGGAGTGGCTTGGGACTATCTCTTTTAGTGGGAGCGCCTACTAC
AACCCGTCCCTCCAGGGTCGAGCCACCATATCGATGGACACGTCCAAGAATCAGTTGTCCC
TGAAGCTGAGGTCTGTGACCGCTGCGGACACGGCCGTCTACTACTGTGCGAGGTATAAGTG
GAACACTTGGTTCGACCCCTGGGGCAGAGGAACCCTGGTCACCGTCTCGAGT

FAT 15 Heavy Chain Amino Acid sequence SEQ ID NO. 48

E V Q L Q Q W G P G L L K P S E T L S L T
C S V S R D S V N R N S N Y W G W I R Q T

P G K K L E W L G T I S F S G S A Y Y N P
S L Q G R A T I S M D T S K N Q L S L K L
R S V T A A D T A V Y Y C A R Y K W N T W
F D P W G R G T L V T V S S

FAT 15 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 16 Heavy Chain DNA Sequence SEQ ID NO. 49

GGGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGATCTCAGCTATTAGTGGTAGTGGTGGTGCCACATTCTACGCA
GACTCCGTGAAGGGCCGTTTCACCATCTCCAGAGACAATTCCAAGGACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAGTCTCTCTATCG
ATGGGAACTCTTTGACTTCTGGGGCCGAGGCACCCTGGTCACCGTATCTTCA

FAT 16 Heavy Chain Amino Acid sequence SEQ ID NO. 50

G V Q L V Q S G G G L V K P G G S L R L S
C A A S G F T F S S Y A M S W V R Q A P G
K G L E W I S A I S G S G G A T F Y A D S
V K G R F T I S R D N S K D T L Y L Q M N
S L R A E D T A V Y Y C A K S L Y R W E L
F D F W G R G T L V T V S S

FAT 16 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 17 Heavy Chain DNA Sequence SEQ ID NO. 51

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTACAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGTTCC
AGGGAAGGGGCTGGAGTGGATCTCAGCTATTAGTGCCAGTAGTGGTAGCACATATTACGCA
GACCCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGC
AAATGAACGGCCTGAGAGCCGAGGACACGGCCTTATATTACTGTGCGAAGTCTCTCTTTCG
GTGGGAGCTATTTGACCTCTGGGGCCAGGGCACCCTGGTCACCGTCTCGAGT

FAT 17 Heavy Chain Amino Acid sequence SEQ ID NO. 52

Q V Q L V E S G G G L V Q P G R S L R L S
C A A S G F T F S S Y A M S W V R Q V P G
K G L E W I S A I S A S S G S T Y Y A D P
V K G R F T I S R D N S K N T L F L Q M N
G L R A E D T A L Y Y C A K S L F R W E L
F D L W G Q G T L V T V S S

FAT 17 Light Chain DNA Sequence SEQ ID NO. 53

TCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAACAAGCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG
AGGCTGACTATTACTGTCATTCCCGGGACAGCAGTGGTAACCATGTGCTTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGT

FAT 17 Light Chain Amino Acid Sequence 54

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N K R P S G I P D R F S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C H S R D S S G N H V L F G G G T K L
T V L G

FAT 18 Heavy Chain DNA Sequence SEQ ID NO. 55

CAGGTGCAGCTACAGCAGTGGGGCCCAGGACTGGTGAAGCCCTCGGAGACCCTGTCACTCA
CGTGCTCTGTGTCTGGTGGCTCCCTCGATGACTTCTTCTGGAGCTGGATCCGGCAGTCCCC
AGGGAAGGGCCTGGAGTGGGTCGCGAGTATCGGCGCCACTGGCAACATACACAGTTCTCCC
TCCCTCAGGAGGCGAGTCACCATGTCAACAGACACGTCCAGAAATCAGTTCTCCCTCAATT
TGACTTCTGTGACCGCTGCGGACACGGCCGTCTATTACTGTGCGAGAGATGGGGAGAGCCC
ACTGGACTTTTACTTCGATTTCTGGGGCCGAGGAACCCTGGTCACCGTCTCGTCC

FAT 18 Heavy Chain Amino Acid sequence SEQ ID NO. 56

Q	V	Q	L	Q	Q	W	G	P	G	L	V	K	P	S	E	T	L	S	L	T
C	S	V	S	G	G	S	L	D	D	F	F	W	S	W	I	R	Q	S	P	G
K	G	L	E	W	V	A	S	I	G	A	T	G	N	I	H	S	S	P	S	L
R	R	R	V	T	M	S	T	D	T	S	R	N	Q	F	S	L	N	L	T	S
V	T	A	A	D	T	A	V	Y	Y	C	A	R	D	G	E	S	P	L	D	F
Y	F	D	F	W	G	R	G	T	L	V	T	V	S	S						

FAT 18 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 19 Heavy Chain DNA Sequence SEQ ID NO. 57

CAGATGCAGCTGGTGCAGTTTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTTT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTATTAAATACTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGACGACACGGCTGTCTATTACTGTGCGAGAGACAGTTGGAT
AAGCGGAAACTTTGACTACTGGGGCAAAGGGACAATTGTGCACAGT

FAT 19 Heavy Chain Amino Acid sequence SEQ ID NO. 58

Q M Q L V Q F G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V I S Y D G S I K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A D D T A V Y Y C A R D S W I S G N
F D Y W G K G T

FAT 19 Light Chain DNA and Amino Acid sequences

Identical to FAT 1 (SEQ ID NO.'s 3 and 4)

FAT 20 Heavy Chain DNA Sequence SEQ ID NO. 59

GAGGTGCAGCTGGTGGAGTTTGGGGGAGGCTTGGTACAGCCGGGGGGGTCCCTGAGACTCT
CCTGTGCAGGCTCTGGATTCAGTTTTAGTCGCTATGCCATAAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTGGTGGAAGCGGTGTTAGCACATTTTACGCA
GGCTCCGTGAGGGGCCGGTTCTCCATCTCCAGAGACAATTCCAAGAACACACTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTATATTACTGTGCGAGAGATTATTTCGA
TATTCTGACTGGTCCCATGGACGTCTGGGGCCGAGGCACCCTTGTCACA

FAT 20 Heavy Chain Amino Acid sequence SEQ ID NO. 60

E V Q L V E F G G G L V Q P G G S L R L S
C A G S G F S F S R Y A I S W V R Q A P G
K G L E W V S A I G G S G V S T F Y A G S
V R G R F S I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A R D Y F D I L T
G P M D V W G R G T L V T

FAT 20 Light Chain DNA Sequence SEQ ID NO. 61

TCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGGCA
GGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATG

AGGCTGACTATTACTGTCATTCCCGGGACAGCAGTGGTAACCATGTGCTTTTCGGCGGAGG
GACCAAGCTGACC

FAT 20 Light Chain Amino Acid Sequence SEQ ID NO. 62

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N N R P S G I P D R F S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C H S R D S S G N H V L F G G G T K L
T

FAT 21 Heavy Chain DNA Sequence SEQ ID NO. 63

GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCCCTGAGAGTCT
CCTGTGCAGCCTCTGGCTTCCCCTTCAGTCACTATGCCATGCACTGGGTCCGCCAGGCCCC
AGGCAAGGGGCTGGAGTGGGTTTCATACATTAGTGGAAGTGGAAGTTATACAGGGTACGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAATTCACTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTA
CTACGGTATGGATGTCTGGGGCAAGGGCACCATTGTCACAGTG

FAT 21 Heavy Chain Amino Acid sequence SEQ ID NO. 64

E V Q L V Q S G G G V V Q P G G S L R V S
C A A S G F P F S H Y A M H W V R Q A P G
K G L E W V S Y I S G S G S Y T G Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R G G H Y Y G M
D V W G K G T I V T V

FAT 21 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 22 Heavy Chain DNA Sequence SEQ ID NO. 65

CAGGTGCAGCTGGTGCAATCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTCGTCTGGCATGATGGCAGTAATGAGTATTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACTCCAAGAACAGCCTATTTCTGC
AAATGAACAACCTGAGCTCCGAGGATACGGCTGTCTATTACTGTGCGAGGGGGTGGTGGTC
GACCAACACCTACTATTTTGACTATTGGGGCAAGGGAACCCTGGTCACCGTCTCGAGT

FAT 22 Heavy Chain Amino Acid sequence SEQ ID NO. 66

Q V Q L V Q S G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V V W H D G S N E Y Y A D S
V K G R F T I S R D N S K N S L F L Q M N
N L S S E D T A V Y Y C A R G W W S T N T
Y Y F D Y W G K G T L V T V S S

FAT 22 Light Chain DNA Sequence SEQ ID NO. 67

GACATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCA
TCACTTGCCAGGCGAGTCAGGACATTAGCAACTATTTAAATTGGTATCAGCAGAAACCAGG
GAAAGCCCCTAAGCTCCTGATCTACGATGCATCCAATTTGGAAACAGGGGTCCCATCAAGG
TTCAGTGGAAGTGGATCTGGGACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCTGAAG
ATATTGCAACATATTACTGTCAACAGTATGATAATCTCCCGATCACCTTCGGCCAAGGGAC
ACGACTGGAGATTAAACGT

FAT 22 Light Chain Amino Acid Sequence SEQ ID NO. 68

D I Q L T Q S P S S L S A S V G D R V T I
T C Q A S Q D I S N Y L N W Y Q Q K P G K
A P K L L I Y D A S N L E T G V P S R F S
G S G S G T D F T F T I S S L Q P E D I A

T Y Y C Q Q Y D N L P I T F G Q G T R L E
I K R

FAT 23 Heavy Chain DNA Sequence SEQ ID NO. 69

CAGGTGCAGCTGCAGGAGTCGGGGGCTGAGGTGAAGAAGTCTGGGGCTTCAGTGAAGGTTT
CCTGCAAGGCATCTGGATACACTTTCACCAGCTACTATATGCACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAATAATCAACCCTAGTGGTGGTAGCACAAGCTACGCG
CAGAAGTTCCAGGGCAGAGTCACCATGACCAGGGACACGTCCACGAGCACAGTCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGATAGTGGCTA
CGATGGGCACGGTATGGACGTCTGGGGCCGAGGAACCCTGGTCACCGTCTCGAGT

FAT 23 Heavy Chain Amino Acid sequence SEQ ID NO. 70

Q V Q L Q E S G A E V K K S G A S V K V S
C K A S G Y T F T S Y Y M H W V R Q A P G
Q G L E W M G I I N P S G G S T S Y A Q K
F Q G R V T M T R D T S T S T V Y M E L S
S L R S E D T A V Y Y C A R D S G Y D G H
G M D V W G R G T L V T V S S

FAT 23 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 25 Heavy Chain DNA sequence SEQ ID NO. 71

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGGAACTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTGATATCATATGATGGAAGTAATAAATACTATGCA
GACTCCGTGGAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGTCGAGGACACGGCTGTTTATTACTGTGCGAGAAGATGGTATGG
TGGCAGTGGTTATTGGGGCCACTTCTACTCCTACATGGACGGCTGGGGCAAAGGGACCAAG
GTCACCGTCTCCTCA

FAT 25 Heavy Chain Amino Acid sequence SEQ ID NO. 72

E V Q L V E S G G G V V Q P G R S L R L S
C A A S G F T F R N Y G M H W V R Q A P G
G L E W V A V I S Y D G S N K Y Y A D S V
E G R F T I S R D N S K N T L Y L Q M N S
L R V E D T A V Y Y C A R R W Y G G S G Y
W G H F Y S Y M D G W G K G T K V T V S S

FAT 25 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 26 Heavy Chain DNA sequence SEQ ID NO. 73

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCCGTGTATTACTGTGCAAGATATTATATTTC
GGGGTGGGGCCAAGGTACCCTGGTCACCGTGTCG

FAT 26 Heavy Chain Amino Acid sequence SEQ ID NO. 74

E V Q L V E S G G G V V Q P G R S L R L S
C A A S G F T F S S Y A M H W V R Q A P G
K G L E W V A V I S Y D G S N K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A R Y Y I S G W G
Q G T L V T V S

FAT 26 Light Chain DNA and Amino Acid sequences

Identical to FAT 2 (SEQ ID NO.'s 7 and 8)

FAT 27 Heavy Chain DNA sequence SEQ ID NO. 75

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCCGTGTATTACTGTGCAAGATATTATGTGTC
TGGGTGGGGCCAAGGTACCCTGGTCACCGTGTCG

FAT 27 Heavy Chain Amino Acid sequence SEQ ID NO. 76

E V Q L V E S G G G V V Q P G R S L R L S
C A A S G F T F S S Y A M H W V R Q A P G
K G L E W V A V I S Y D G S N K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A R Y Y V S G W G
Q G T L V T V S

FAT 27 Light Chain DNA and Amino Acid sequences

Identical to FAT 2 (SEQ ID NO.'s 7 and 8)

FAT 28 Heavy Chain DNA Sequence SEQ ID NO. 77

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGAGTCCCTTAGACTCT
CCTGTGCAGCCTCTGGATTCACTTTCAGTAACGCCTGGATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTGGCCGTATTAAAAGCAAAACTGATGGTGGGACAACAGAC
TACGCTGCACCCGTGAAAGGCAGATTCACCATCTCAAGAGATGATTCAAAAAACACGCTGT
ATCTGCAAATGAACAGCCTGAAAACCGAGGACACGGCCGTGTATTACTGTGCAAGATGGGG
TCCTCCGGTGTATGCTAAGCCTTGGGGCCAAGGTACCCTGGTCACCGTGTCG

FAT 28 Heavy Chain Amino Acid sequence SEQ ID NO. 78

E V Q L V E S G G G L V K P G E S L R L S
C A A S G F T F S N A W M S W V R Q A P G
K G L E W V G R I K S K T D G G T T D Y A
A P V K G R F T I S R D D S K N T L Y L Q
M N S L K T E D T A V Y Y C A R W G P P V
Y A K P W G Q G T L V T V S

FAT 28 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 29 Heavy Chain DNA Sequence SEQ ID NO. 79

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGCGCTGTCTCTGGTTACTCCATCAGCAGTGGTTACTACTGGGGCTGGATCCGGCAGCC
CCCAGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATCATAGTGGGAGCACCTACTACAAC
CCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGA
AGCTGAGCTCTGTGACCGCCGCAGACACGGCTGTGTATTACTGTGCAAGAGTGAATAGGTA
TGGTTCTCCTTAGACGTGGGGCCAAGGTACCCTGGTCACCGTGTCG

FAT 29 Heavy Chain Amino Acid sequence SEQ ID NO. 80

Q V Q L Q E S G P G L V K P S E T L S L T
C A V S G Y S I S S G Y Y W G W I R Q P P
G K G L E W I G S I Y H S G S T Y Y N P S
L K S R V T I S V D T S K N Q F S L K L S
S V T A A D T A V Y Y C A R V N R Y G S P
B T W G Q G T L V T V S

FAT 29 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 30 Heavy Chain DNA sequence SEQ ID NO. 81

GAGGTGCAGCTGGTGGAGACCGGAGCAGAGGTGAAAAAGCCCGGCGAGTCTCTGAAGATTT
CCTGTGAGATTTCTGGGTACACCTTTACCGACTACTGGATCGCCTGGGTGCGCCAGATGCC
CGGGAAAGGCTTGGAGTGGATGGGTATTATCTATCCTGGTGACTCGGATGCCAGATACAGC
CCGTCCTTCGAAGGCCAGGTCACCATGTCAGCCGACGAGTCCCTCAGCACCGTCTACCTCC
AATGGAGCAGCCTGAAGCCCTCGGACAGCGCCATGTATTTCTGTGCGCGGCCCCATTACCC
CATGACTACGGATGATGCTTTTGATATTTGGGGCAAAGGAACCCTGGTCACCGTCTCGAGT

FAT 30 Heavy Chain Amino Acid sequence SEQ ID NO. 82

E V Q L V E T G A E V K K P G E S L K I S
C E I S G Y T F T D Y W I A W V R Q M P G
K G L E W M G I I Y P G D S D A R Y S P S
F E G Q V T M S A D E S L S T V Y L Q W S
S L K P S D S A M Y F C A R P H Y P M T T
D D A F D I W G K G T L V T V S S

FAT 30 Light Chain DNA sequence SEQ ID NO. 83

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC
AGGCAAAGCCCCCAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTCCTCTTCGG
AACTGGGACCAAGCTGACCGTCCTAGGTGCGGCCGCACATCATCATCACCATCAC

FAT 30 Light Chain Amino Acid sequence SEQ ID NO. 84

Q S V L T Q P P S V S A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A P K L M I Y D V S K R P S G V P D R F
S G S K S G N S A S L D I S G L Q S E D E

A D Y Y C A A W D D S L S E F L F G T G T
K L T V L G

FAT 31 Heavy Chain DNA Sequence SEQ ID NO. 85

GAGGTGCAGCTGGTGGAGTCTGGGGGGGGCTTGGTACAGCCTGGCAGGTCCCTGAGACTCT
CCTGTGGAGGCTCTGGATTCAGTTTTGATGAATATGCCATGCACTGGGTCCGCCAAGCTCC
AGGCAAGGGCCTGGAGTGGGTCCCAGGTATTAATTGGAATGGTGTTAGTTTGGCCTATGCG
GACTCTGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGCATCTGC
AAATGAACAGTTTGGGGACTGAGGACACGGCCTTCTATTACTGTGCAAAAGCTGCCATAGC
CTCCTTAGGCAATTGTACGAGTGCCAGTTGCTATAACGGTGCTTTTGACATCTGGGGCCGG
GGGACAATGGTCACCGTC

FAT 31 Heavy Chain Amino Acid sequence SEQ ID NO. 86

E V Q L V E S G G G L V Q P G R S L R L S
C G G S G F S F D E Y A M H W V R Q A P G
K G L E W V P G I N W N G V S L A Y A D S
V K G R F T I S R D N A K N S L H L Q M N
S L G T E D T A F Y Y C A K A A I A S L G
N C T S A S C Y N G A F D I W G R G T M V
T V

FAT 31 Light Chain DNA Sequence SEQ ID NO. 87

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC
AGGCAAAGCCCACAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTC

FAT 31 Light Chain Amino Acid Sequence SEQ ID NO. 88

Q S V L T Q P P S V S A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A H K L M I Y D V S K R P S G V P D R F
S G S K S G N S A S L D I S G L Q S E D E
A D Y Y C A A W D D S L S E F L F G T G T
K L T V

FAT 32 Heavy Chain DNA sequence SEQ ID NO. 89

GAAGTGCAGCTGGTGCAGTTGGAGCAGGAGGTGAAAAAGCCGGGGGGAGTTTGGAAGATCT
CCTGTAAGGGTTCTGGATACAGCTTTAAACCCCAATGGCTCGGCTGGGTGCGCCAGATGCC
CGGGAAAGGCCTGGAGTGTATGGGAATCATCTATCCTGGTGACTCTGATACCAAATACAGC
CCGTCCTTCCAAGGCCAGGTCACCCTCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGC
AGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTATACCAGCACAGACGATGG
CTACAATTTTGCTTTTGATATCTGGGGCAGAGGAACCCTGGTCACCGTCTCGAGT

FAT 32 Heavy Chain Amino Acid sequence SEQ ID NO. 90

E V Q L V Q L E Q E V K K P G G V W K I S
C K G S G Y S F K P Q W L G W V R Q M P G
K G L E C M G I I Y P G D S D T K Y S P S
F Q G Q V T L S A D K S I S T A Y L Q W S
S L K A S D T A M Y Y Y T S T D D G Y N F
A F D I W G R G T L V T V S S

FAT 32 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 33 Heavy Chain DNA sequence SEQ ID NO. 91

CAGGTCACCTTGAAGGAGTCTGGGGGAGACTTGGTCAGGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGACCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCATACATTACTAATAGTGGTAATACCATAGACTACGCA
GACTCTGTGCAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTCC

AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGACTGGTTCCGGGGA
CCTTGACCACTGGGGCCAGGGCACCCTGGTCACCGTCTCGAGT

FAT 33 Heavy Chain Amino Acid sequence SEQ ID NO. 92

Q	V	T	L	K	E	S	G	G	D	L	V	R	P	G	G	S	L	R	L	S
C	A	A	S	G	F	T	F	S	D	Y	Y	M	T	W	I	R	Q	A	P	G
K	G	L	E	W	V	S	Y	I	T	N	S	G	N	T	I	D	Y	A	D	S
V	Q	G	R	F	T	I	S	R	D	N	A	K	N	S	L	Y	L	Q	M	N
S	L	R	A	E	D	T	A	V	Y	Y	C	A	T	G	S	G	D	L	D	H
W	G	Q	G	T	L	V	T	V	S	S										

FAT 33 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 34 Heavy Chain DNA sequence SEQ ID NO. 93

GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTATTACATGAGCTGGATCCGCCAGACTCC
AGGGAAGGGTCTGGAATGGATTTCATACATTAGTGATAACGGTAAAACCATATACTACGGA
GACTCTGTGGAGGGCCGATTCACCATCTCCAGGGACAACGCCAACCGCTCACCGGATCTGC
AAATGAACAGCCTGAGAGCCGACGACACGGCCGTGTATTTCTGTGCGAGAAGCATGGGCAC
TGGCTGGTATGTTAGCTACCCTGACTTCTGGGGCAAAGGCACCACGGTCACCGTCTCCTCA

FAT 34 Heavy Chain Amino Acid sequence SEQ ID NO. 94

E	V	Q	L	V	Q	S	G	G	G	L	V	K	P	G	G	S	L	R	L	S
C	A	A	S	G	F	T	F	S	D	Y	Y	M	S	W	I	R	Q	T	P	G
K	G	L	E	W	I	S	Y	I	S	D	N	G	K	T	I	Y	Y	G	D	S
V	E	G	R	F	T	I	S	R	D	N	A	N	R	S	P	D	L	Q	M	N

S L R A D D T A V Y F C A R S M G T G W Y
V S Y P D F W G K G T T V T V S S

FAT 34 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 35 Heavy Chain DNA Sequence SEQ ID NO. 95

CAGGTCACCTTGAAGGAGTCTGGGGGAGGCTTGGTCAAGGCTGGGGGTTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCAGAGTGGAACCGCCAATTACAACCCC
TCCCTCAAGAGTCGAGTCACCTTGTCAGTGGACAGGTCCGCGAACCAGTTCTCGCTGAAGC
TGACCTCTGTGACCGCCGCGGACACGGCCGTGTATTATTGTGCAAGAGATACGGTCGGGGA
CTATGATAGTGGTGGCTATTACTACTCTGACTCCTGGGGCAAAGGCACCCCGGTCACCGTC
TCCTCA

FAT 35 Heavy Chain Amino Acid sequence SEQ ID NO. 96

Q V T L K E S G G G L V K A G G S L R L S
C A A S G F T F S D Y Y M S W I R Q A P G
K G L E W I G E I N Q S G T A N Y N P S L
K S R V T L S V D R S A N Q F S L K L T S
V T A A D T A V Y Y C A R D T V G D Y D S
G G Y Y Y S D S W G K G T P V T V S S

FAT 35 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 36 Heavy Chain DNA Sequence SEQ ID NO. 97

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAAACTACGCA

TAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGG
AGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATGGGGTTCT
AGACTACTACTATGGTATGGACGTCTGGGGCCGGGGAACCCTGGTCACCGTCTCGAGTGGT
GGAGGCGGTT

FAT 36 Heavy Chain Amino Acid sequence SEQ ID NO. 98

E V Q L V E S G A E V K K P G A S V K V S
C K A S G Y T F T S Y G I S W V R Q A P G
Q G L E W M G W I S A Y N G N T N Y A B K
L Q G R V T M T T D T S T S T A Y M E L R
S L R S D D T A V Y Y C A R D G V L D Y Y
Y G M D V W G R G T L V T V S S

FAT 36 Light Chain DNA and Amino Acid sequence

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 37 Heavy Chain DNA Sequence SEQ ID NO. 99

CAGGTCCAGCTGGAGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCACCAATTCTATCAACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTGTCTTTGATGCATCAAATTACGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAGTCCACGAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTTCCTCCAACTATTACTA
TGATAGTAGTGGTTATTACTTATATGATGCTTTTGATATCTGGGGCCGAGGCACCCTGGTC
ACCGTCTCCTCA

FAT 37 Heavy Chain Amino Acid sequence SEQ ID NO. 100

Q V Q L E Q S G A E V K K P G S S V K V S
C K A S G G T F S T N S I N W V R Q A P G
Q G L E W M G G I I P V F D A S N Y A Q K
F Q G R V T I T A D E S T S T A Y M E L S

S L R S E D T A V Y Y C S S N Y Y Y D S S
G Y Y L Y D A F D I W G R G T L V T V S S

FAT 37 Light Chain DNA Sequence SEQ ID NO. 101

GATGTTGTGATGACTCAGTCTCCATCTTCCGTGTCTGCGGCCCCAGGACAGAAGGTCACCA
TTTCCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCT
GACCGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGT
CTGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTT
CGGAACTGGGACCAAGCTGACCGTCCTAGGT

FAT 37 Light Chain Amino Acid Sequence SEQ ID NO. 102

D V V M T Q S P S S V S A A P G Q K V T I
S C S G S T S N I G N N Y V S W Y Q Q H P
G K A P K L M I Y D V S K R P S G V P D R
F S G S K S G N S A S L D I S G L Q S E D
E A D Y Y C A A W D D S L S E F L F G T G
T K L T V L

FAT 38 Heavy Chain DNA Sequence SEQ ID NO. 103

GAGGTGCAGCTGGTGGAGACCGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGATACACCTTCACCAGTTATGATTTCAACTGGGTGCGACAGGCCAC
TGGACAAGGGCTTGAGTGGATGGGATGGATGAACCCTAACAGTGGTGACACAGGCTACGCA
CAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTACATGG
AGCTGAGGAGCCTGAGACCTGACGACTCGGCCGTGTATTACTGTGCGGTGTGGCCCGATTG
TAGTGGTACCAGCTGCTATTCTCCTAACTACTGGGGGAAAGGGACCACGGTCACCGTCTCC
TCA

FAT 38 Heavy Chain Amino Acid sequence SEQ ID NO. 104

E V Q L V E T G A E V K K P G S S V K V S
C K A S G Y T F T S Y D F N W V R Q A T G

Q G L E W M G W M N P N S G D T G Y A Q K
F Q G R V T M T E D T S T D T A Y M E L R
S L R P D D S A V Y Y C A V W P D C S G T
S C Y S P N Y W G K G T T V T V S S

FAT 38 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 39 Heavy Chain DNA Sequence SEQ ID NO. 105

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCGGCAACTATGGTATCGACTGGGTGCGACTGGCCCC
TGGACAAGGACTTGAGTGGATGGGAGGGATCATCCCTCTTTTTCGTACAACAAATTACGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACGGCTTACATGG
AGATGAGCAGTCTGAGATCTGACGACACGGCCGTGTATTATTGTGCGAGATATGATGCTCG
TGGTTATTATTATTTGGACTTCTGGGGCAAGGGCACCCTGGTCACCGTCTCGAGT

FAT 39 Heavy Chain Amino Acid sequence SEQ ID NO. 106

E V Q L V E S G A E V K K P G S S V K V S
C K A S G G T F G N Y G I D W V R L A P G
Q G L E W M G G I I P L F R T T N Y A Q K
F Q G R V T I T A D E S T S T A Y M E M S
S L R S D D T A V Y Y C A R Y D A R G Y Y
Y L D F W G K G T L V T V S S

FAT 39 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 40 Heavy Chain DNA Sequence SEQ ID NO. 107

CAGGTGCAGCTGCAGGAGTCGGGTCCAAGACTGGTGAAGCCTTCGGGGACCCTGTCCCTCA
CATGCGCTGTCTCTGGTGCCTCCATCTATAGCACTAATTTCTACAGTTGGGTCCGCCAGCC
CCCAGGGAAGGGCCTGGAGTGGATTGGAGAAATCTCTCTTAGTGGGGGCATCAACTACAAC
CCGTCCCTCAGCAGTCGAGTCACCATATCAATGGACAAGTCCAAGAACCAGATCTCCCTGC
AGATGACCTCTGTGACCGCCGCGGACACGGCCATGTATTACTGTGCGAGGGGGTACAACTG
GCACTACGATGATGCTTTTGATATCTGGGGCCAGGGGACAATGGTCACCGTCTCGAGT

FAT 40 Heavy Chain Amino Acid sequence SEQ ID NO. 108

Q V Q L Q E S G P R L V K P S G T L S L T
C A V S G A S I Y S T N F Y S W V R Q P P
G K G L E W I G E I S L S G G I N Y N P S
L S S R V T I S M D K S K N Q I S L Q M T
S V T A A D T A M Y Y C A R G Y N W H Y D
D A F D I W G Q G T M V T V S S

FAT 40 Light Chain DNA and Amino Acid sequences

Identical to FAT 11 (SEQ ID NO.'s 35 and 36)

FAT 41 Heavy Chain DNA Sequence SEQ ID NO. 109

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGAGGCTAGCCT
CAACCTATGGCCGGACCCGACGTGGGCTTTTGATATCTGGGGCCGAGGCACTCTGGTCACC
GTCTCGAGT

FAT 41 Heavy Chain Amino Acid sequence SEQ ID NO. 110

Q V Q L V Q S G A E V K K P G S S V K V S
C K A S G G T F S S Y A I S W V R Q A P G
Q G L E W M G G I I P I F G T A N Y A Q K
F Q G R V T I T A D E S T S T A Y M E L S
S L R S E D T A V Y Y C A R E A S L N L W
P D P T W A F D I W G R G T L V T V S S

FAT 41 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 42 Heavy Chain DNA Sequence SEQ ID NO. 111

CAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
TCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGGTAGAGCAGC
AGCTGACAAAACTGACTACTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCA

FAT 42 Heavy Chain Amino Acid sequence SEQ ID NO. 112

Q V Q L V Q S G A E V K K P G S S V K V F
C K A S G G T F S S Y A I S W V R Q A P G
Q G L E W M G G I I P I F G T A N Y A Q K
F Q G R V T I T A D E S T S T A Y M E L S
S L R S D D T A V Y Y C A R G R A A A D K
T D Y W G Q G T L V T V S S

FAT 42 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 44 Heavy Chain DNA Sequence SEQ ID NO. 113

GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCA
CAAGAGCTTCAACAGGGGAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACA
TGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGCGTATTACTGTGCGAGAAAGGGGCT
AGACCGAACCTACTACATGGACGTCTGGGGGCAGGTCGAGTCCTGGGGCAGGGGGACCACG
GTCACCGTCTCTTCA

FAT 44 Heavy Chain Amino Acid sequence SEQ ID NO. 114

E V Q L V Q S G A E V K K P G S S V K V S
C K A S G G T F S S Y A I S W V R Q A P G
Q G L E W M G G I I P I F G T A N Y A Q E
L Q Q G R V T I T A D E S T S T A Y M E L
S S L R S E D T A A Y Y C A R K G L D R T
Y Y M D V W G Q V E S W G R G T T V T V S
S

FAT 44 Light Chain DNA Sequence SEQ ID NO. 115

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC
AGGCAAAGCCCCCGAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTCCTA

FAT 44 Light Chain Amino Acid Sequence SEQ ID NO. 116

Q S V L T Q P P S V S A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A P E L M I Y D V S K R P S G V P D R F
S G S K S G N S A S L D I S G L Q S E D E

35

A D Y Y C A A W D D S L S E F L F G T G T
K L T V L

FAT 45 Heavy Chain DNA sequence SEQ ID NO. 117

CAGGTCAACTTAAGGGAGTCTGGGGGAGGCTTGGTACAGCCAGGAGGGTCCCTGAGACTCT
CCTGCGTAGCCTCTGGATTCACCTTGAGTAATTTTGACCTGAATTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGCTTTCATACATCAGTAGCAGTGGTTCCACAATATCCTACGCA
GACTCTGTGAGGGGCCGATTCACCATCTCCAGAGACCACGTCAAGAACTCACTATCTCTGC
AAATGAAGAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAAAGGGGGGAGCAG
CCCCGCGGGAGTCGCAGACTACTGGGGCCAAGGCACCCTGGTCACCGTCTCGAGT

FAT 45 Heavy Chain Amino Acid sequence SEQ ID NO. 118

Q V N L R E S G G G L V Q P G G S L R L S
C V A S G F T L S N F D L N W V R Q A P G
K G L E W L S Y I S S S S G S T I S Y A D S
V R G R F T I S R D H V K N S L S L Q M K
S L R A E D T A V Y Y C A K G G S S P A G
V A D Y W G Q G T L V T V S S

FAT 45 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 46 Heavy Chain DNA sequence SEQ ID NO. 119

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATGTGC
AAATGAACAGTCTGAGAGCCGAGGACACGGCCTTGTATCACTGTGCGAGCTCTATGATCGA
AGGTGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCGAGT

FAT 46 Heavy Chain Amino Acid sequence SEQ ID NO. 120

E V Q L V E S G G G V V R P G G S L R L S
C A A S G F T F D D Y G M S W V R Q A P G
K G L E W V S G I N W N G G S T G Y A D S
V K G R F T I S R D N A K N S L Y V Q M N
S L R A E D T A L Y H C A S S M I E G A F
D I W G Q G T M V T V S S

FAT 46 Light Chain DNA and Amino Acid sequences

Identical to FAT 44 (SEQ ID NO.'s 115 and 116)

FAT 48 Heavy Chain DNA Sequence SEQ ID NO. 121

GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGAGGTTCCCGGAAACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCAGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCGCATACTACGCA
GACTCCGTGAAGGGCCGGTTCACCATTTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAAAGCCTATAGCAG
TGAAGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA

FAT 48 Heavy Chain Amino Acid sequence SEQ ID NO. 122

E V Q L V Q S G G G V V Q P G G S R K L S
C A A S G F T F S S Y A M S W V R Q A P G
K G Q E W V S A I S G S G G S A Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A K A Y S S E D Y
W G Q G T L V T V S S

FAT 48 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 49 Heavy Chain DNA Sequence SEQ ID NO. 123

CAGGTCACCTTGAAGGAGTCTGGGGGAGGCTTGTTCCAGCCTGGGGGTTCCCTGAGACTCT
CCTGTGCAGCTTCTGGATTCACCTTTAGTAGCAATTGGATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCAACTATCAGTGATAGTGGTGGTCTCACACATTCCGCA
GACTCCCTGAAGGGCCGGGCCACCGTCCCCAGAGACAATTCCGAGAACACGATGTATCTGG
AACTGCGCGGCCTGAGAGCCGACGACTCGGCCAATTATTACTGTGCTAGAGGTCTTACCGT
TTTTGGCGTAGTCAATGCTCTTGATGTCTGGGGCAAGGGAACCCTGGTCACCGTCTCCTCA

FAT 49 Heavy Chain Amino Acid sequence SEQ ID NO. 124

Q V T L K E S G G G L F Q P G G S L R L S
C A A S G F T F S S N W M S W V R Q A P G
K G L E W V S T I S D S G G L T H S A D S
L K G R A T V P R D N S E N T M Y L E L R
G L R A D D S A N Y Y C A R G L T V F G V
V N A L D V W G K G T L V T V S S

FAT 49 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 50 Heavy Chain DNA Sequence SEQ ID NO. 125

CAGGTGCAGCTGCAGCAGTCGGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCA
CATGCACCGTCTCAGGGTTCTCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCC
AGGAAAGGGTCTGGAGTGGCTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCA
GCTCTCAAATCCAGACCGAGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAA
TGAACAGTCTGCACACTGATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAG
GCTTGACTACTGGGGCCGAGGGACAATGGTCACGTCT

FAT 50 Heavy Chain Amino Acid sequence SEQ ID NO. 126

Q V Q L Q Q S G P G L V A P S Q S L S V T
C T V S G F S L T G Y G V N W V R Q P P G
K G L E W L G M I W G D G N T D Y N S A L
K S R P S I S K D N S K S Q V F L K M N S
L H T D D T A R Y Y C A R E R D Y R L D Y
W G R G T M V T S

FAT 50 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 52 Heavy Chain DNA Sequence SEQ ID NO. 127

CAGGTACAGCTGCAGCAGTCAGGAACTGAGGTGAAGAGACCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGACTTCTGGCTACACCTTTCCCAGTTATGGTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATCAACTGTTACAATGGTAATACAAACTATGCA
CAGAGCTTCCAGGACAGAGTCACCATGACCGCAGACACATCCACGACTACAGCCTACATGG
AGGTGAGGAAGCTGAAGTCTGACGACACGGCCGTGTATTTCTGTGCGAGGTCGCTCGTCCC
AACCAACTGTGACAACTGGGGCCGAAGCACCCTGGTCACCGTCTCCTCA

FAT 52 Heavy Chain Amino Acid sequence SEQ ID NO. 128

Q V Q L Q Q S G T E V K R P G A S V K V S
C K T S G Y T F P S Y G I S W V R Q A P G
Q G L E W M G W I N C Y N G N T N Y A Q S
F Q D R V T M T A D T S T T T A Y M E V R
K L K S D D T A V Y F C A R S L V P T N C
D N W G R S T L V T V S S

FAT 52 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 53 Heavy Chain DNA Sequence SEQ ID NO. 129

CAGGTGCAGCTGTTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGACTCTCTGAAGATCT
CCTGTAAGGCTTCTGGATACAACTTTCCCAACTCTTGGATCGGCTGGGTGCGCCAGATGCC
CGGGAAGGGCCTGGAGTACATGGGGCTCATCTATCCTGGTGACTCTGACACCAAATACAGC
CCGTCCTTCCAAGGCCAGGACACCATGTCAGTCGACAAGTCCGTCAGCACTGCCTACTTGC
AATGGAGCAGTCTGAGGCCCTCGGACAGCGCCGTGTATTTTTGTGCGAGACATGACGTGGG
ATATTGCAGTAGTTCCAACTGCGCAAGGAGGCCTGAATACTTCCAGCATTGGGGCCGAGGA
ACCCTGGTCACCGTCTCCTCA

FAT 53 Heavy Chain Amino Acid sequence SEQ ID NO. 130

Q V Q L L Q S G A E V K K P G D S L K I S
C K A S G Y N F P N S W I G W V R Q M P G
K G L E Y M G L I Y P G D S D T K Y S P S
F Q G Q D T M S V D K S V S T A Y L Q W S
S L R P S D S A V Y F C A R H D V G Y C S
S S N C A R R P E Y F Q H W G R G T L V T
V S S

FAT 53 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 54 Heavy Chain DNA Sequence SEQ ID NO. 131

CAGATGCAGCTGGTGCAGTTTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCAGTTATGCTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGTTCATCCCTATCTTTGATACAGCAAACTACGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGATGCCTCCAT
ACCCGATGATACATGGGACTACTGGGGCAGAGGGACAATGGTCACGGTCTCGAGT

FAT 54 Heavy Chain Amino Acid sequence SEQ ID NO. 132

Q M Q L V Q F G A E V K K P G S S V K V S
C K A S G G T F S S Y A I S W V R Q A P G
Q G L E W M G G F I P I F D T A N Y A Q K
F Q G R V T I T A D E S T S T A Y M E L S
S L R S E D T A V Y Y C A R D A S I P D D
T W D Y W G R G T M V T V S S

FAT 54 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 55 Heavy Chain DNA Sequence SEQ ID NO. 133

CAGGTGCAGCTGCAGGAGTCCGGCCCAGGAATGGGTGGAGCCCTTTCGGAGACCCTGTCCC
TCACCTGCTCTGTCTCTGGTGACTCCATCTCCAGTGGTGGTTACTCCTGGAGCTGGATCCG
GCAGCCATCAGGGAAGGGACTGGAGTGGGTCTCTTCCATTAGTAGCAATAATCGGTTCATA
TACTACGCAGACTCAGTGCAGGGCCGATTCACCATCTCCAGAGACAACCCCAAAAACTCAC
TGTCTCTGCAAATGAGCAGTCTGAGAGCCGAGGACACGGCTGTCTACTACTGTGCGAGAGG
TCAGCGCCTGTACATTGACTCCTGGGGCCGAGGCACCCTGGTCACGGTCTCCTCA

FAT 55 Heavy Chain Amino Acid sequence SEQ ID NO. 134

Q V Q L Q E S G P G M G G A L S E T L S L
T C S V S G D S I S S G G Y S W S W I R Q
P S G K G L E W V S S I S S N N R F I Y Y
A D S V Q G R F T I S R D N P K N S L S L
Q M S S L R A E D T A V Y Y C A R G Q R L
Y I D S W G R G T L V T V S S

FAT 55 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 56 Heavy Chain DNA Sequence SEQ ID NO. 135

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCTTTGCAGACCCCCTCACTCA
CCTGTGCCATTTCCGGGGACAGTGTCGCTAGTAACAGTGCTGCTTGGAACTGGATCAGGCA
GTCCCCTTCGAGAGGCCTTGAGTACCTGGGAAGGACATACTACAGGTCCAGGTGGTACACT
GAATATGCAGTGCCTGTGAAAAGTCGCATAACCATCAACCCCGACACATCCAGGAACCAGT
ACTCCCTGCAGCTGAATTCTGTGACTCCCGAGGACACGGCCGTGTATTACTGTGCAAGAGA
CGGTTCGCTGGGGCTTGATGCTCTTGATATCTGGGGCCAAGGAACCCTGGTCACCGTCT

FAT 56 Heavy Chain Amino Acid sequence SEQ ID NO. 136

Q V Q L Q Q S G P G L V K P L Q T P S L T
C A I S G D S V A S N S A A W N W I R Q S
P S R G L E Y L G R T Y Y R S R W Y T E Y
A V P V K S R I T I N P D T S R N Q Y S L
Q L N S V T P E D T A V Y Y C A R D G S L
G L D A L D I W G Q G T L V T V

FAT 56 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 57 Heavy Chain DNA sequence SEQ ID NO. 137

CAAGTCACCTTGAAGGAGTCTGGGGCTGAGGTGAAGAAGTCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGACAGCTTCAATAGCCACGCTATCAACTGGGTGCGACAGGGCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTTTGTTTGGTACTGCAAAGTACGCA
CAGCAGTTCCAGGGCAGAGTCACAATTACCGCGGACGAATCCACGAACCCAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTTTTACTGTGCGAGAGGGAAGTACGC
TGGTAATTCCGGTCGGCACGGTATGGACGTCTGGGGCCAGGGGACAATGGTCACGGTCTCG
AGT

FAT 57 Heavy Chain Amino Acid sequence SEQ ID NO. 138

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Q V T L K E S G A E V K K S G S S V K V S
C K A S G D S F N S H A I N W V R Q G P G
Q G L E W M G G I I P L F G T A K Y A Q Q
F Q G R V T I T A D E S T N P A Y M E L S
S L R S E D T A V F Y C A R G K Y A G N S
G R H G M D V W G Q G T M V T V S S

FAT 57 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 58 Heavy Chain DNA Sequence SEQ ID NO. 139

GAGGTGCAGCTGGTGCGGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTAGTAGTTACACAAACTACGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGATCGTGATAG
TAGTGGTTATCACATCTGGGGCCAGGGGACAATGGTCACGGTCTCTTCA

FAT 58 Heavy Chain Amino Acid sequence SEQ ID NO. 140

E V Q L V R S G G G L V K P G G S L R L S
C A A S G F T F S D Y Y M S W I R Q A P G
K G L E W V S Y I S S S S S S Y T N Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R D R D S S G Y
H I W G Q G T M V T V S S

FAT 58 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 59 Heavy Chain DNA Sequence SEQ ID NO. 141

CAGGTCACCTTGAAGGAGTCTGGGGGAGAATTGGTCCAGCCTGGGGGGTCCCCGAGACTCT
CCTGTTCAGCCTCTGGATTCACCTTCAGTAGTCTTGCTATGCACTGGGTCCGCCAGGCTCC
AGGGAGGGGACTGGAATATGTTTCCATTAGTAATGGTGATGGGACTAGCACAACCTACGCA
GACTCCGTGAAGGGCAGATTCACCACATCCAGAGACAATTCCAAGAACACGATGTATCTTC
AAATGAACAGTCTGAGACCTGAGGACACGGCTGTGTATTACTGTGTGAGAGATGTTTACGG
CATGGACGTCTGGGGCAGAGGCACCCTGGTCACCGTCTCCTCA

FAT 59 Heavy Chain Amino Acid sequence SEQ ID NO. 142

Q	V	T	L	K	E	S	G	G	E	L	V	Q	P	G	G	S	P	R	L	S
C	S	A	S	G	F	T	F	S	S	L	A	M	H	W	V	R	Q	A	P	G
R	G	L	E	Y	V	S	I	S	N	G	D	G	T	S	T	T	Y	A	D	S
V	K	G	R	F	T	T	S	R	D	N	S	K	N	T	M	Y	L	Q	M	N
S	L	R	P	E	D	T	A	V	Y	Y	C	V	R	D	V	Y	G	M	D	V
W	G	R	G	T	L	V	T	V	S	S										

FAT 59 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 60 Heavy Chain DNA sequence SEQ ID NO. 143

CAGGTCACCTTGAAGGAGTCTGGGGCTGAGGTGGAGAAACCTGGGGCCTCAGTGAAGGTTT
CCTGCAAGGCCTCTGGATACAGTTTCACTAACTATGCTATTCATTGGGTGCGCCAGGCCCC
CGGACAAAGGCTTGAGTGGATGGGATGGATCAACGCTGGCAATGGTGACACAACATATTCA
CAGAGGTTCCAGGGCAGATCAACATGACCAAGGACACATCTACAGAGACAGCCTACATGG
AGCTGCGCAGCCTGAGACCTGAGGACACGGCCGTGTATTATTGTACGCGAAGGAGCGGGGA
TGTGGATACAGATATGATAACCAGTGATGCTGTTGATATCTGGGGCAAGGGAACCCTGGTC
ACCGTCTCCTCA

FAT 60 Heavy Chain Amino Acid sequence SEQ ID NO. 144

Q V T L K E S G A E V E K P G A S V K V S
C K A S G Y S F T N Y A I H W V R Q A P G
Q R L E W M G W I N A G N G D T T Y S Q R
F Q G R V N M T K D T S T E T A Y M E L R
S L R P E D T A V Y Y C T R R S G D V D T
D M I T S D A V D I W G K G T L V T V S S

FAT 60 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 61 Heavy Chain DNA Sequence SEQ ID NO. 145

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTTTGGAGGCACCTTCGGCAGATATGCAATCACCTGGGTGCGGCAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATGTTCGGTACAACGAAATCCGCA
CAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AGTTGAGCAGCTTGAGATCTGAGGACACGGCCGCGTATTACTGTGCGAGAGATTACTATGA
TAACGGGGCGACTAACTTTGATTACTGGGGCAAGAGGACAATGGTCACCGTCTCTTCA

FAT 61 Heavy Chain Amino Acid sequence SEQ ID NO. 146

E V Q L V E S G A E V K K P G S S V K V S
C K A F G G T F G R Y A I T W V R Q A P G
Q G L E W M G G I I P M F G T T K S A Q K
F Q G R V T I T A D E S T S T A Y M E L S
S L R S E D T A A Y Y C A R D Y Y D N G A
T N F D Y W G K R T M V T V S S

FAT 61 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 62 Heavy Chain DNA sequence SEQ ID NO. 147

CAGGTGCAGCTGCAGCAGTCAGGGGGGGGCTTGGTCCAGCCGGGGGGGTCCCTGAGAATCT
CCTGTGCAGCCTCTGGATTCACCTCCAGTGACTACTACATGAGATGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCAAACATTAGTTCTAGTGGTAGTAGCATATACTACGCA
GACTACATGTTCAGGACTAATTCAACACTTACTGTAGACACATCCTCCAGTACAGCCTACA
TGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTTCAAGAGGGGACGG
CAGTGATTATTATGCTATGGACTACTGGGGCAGAGGAACCCTGGTCACCGTCTCCTCA

FAT 62 Heavy Chain Amino Acid sequence SEQ ID NO. 148

Q	V	Q	L	Q	Q	S	G	G	G	L	V	Q	P	G	G	S	L	R	I	S
C	A	A	S	G	F	T	S	S	D	Y	Y	M	R	W	I	R	Q	A	P	G
K	G	L	E	W	V	S	N	I	S	S	S	G	S	S	I	Y	Y	A	D	Y
M	F	R	T	N	S	T	L	T	V	D	T	S	S	S	T	A	Y	M	Q	L
S	S	L	T	S	E	D	S	A	V	Y	Y	C	S	R	G	D	G	S	D	Y
Y	A	M	D	Y	W	G	R	G	T	L	V	T	V	S	S					

FAT 62 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 63 Heavy Chain DNA sequence SEQ ID NO. 149

CAGGTCACCTTGAAGGAGTTTGAGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATCTAATTTCAGTAGTTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGACAGTTATATCATATGATGGAAGTGATAAATACTATGCA
GACTCCGTGAAGGGCCAATTCATCGCCTCCAGAGACAATTCCAAGAACTCCCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTTTATTACTGTGCGAGAGATGGGACAAC
TAGAACGACGGCCACGGACTACATGGACGTCTGGGGGAAAGGGACCACGGTCACCGTCTCT
TCT

FAT 63 Heavy Chain Amino Acid sequence SEQ ID NO. 150

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Q V T L K E F E G G V V Q P G R S L R L S
C A A S G S N F S S Y G M H W V R Q A P G
K G L E W V T V I S Y D G S D K Y Y A D S
V K G Q F I A S R D N S K N S L Y L Q M N
S L R A E D T A V Y Y C A R D G T T R T T
A T D Y M D V W G K G T T V T V S S

FAT 63 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 64 Heavy Chain DNA sequence SEQ ID NO. 151

CAGGAGCAGATGCAGGAGTCGGGGGGTGAGGTGAAGAAGCCTGGGTCCTCGGTGGGGGTGT
CCTGCAAGGCTTCAGGAGGCACATTCAGCAGCTATGCTATCAGCTGGATGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATGAACCCTAACAGTGGTAACACAGGCTATGCA
CAGAAGTTCCAGGGCAGAGTCACCATGACCAGGAACACCTCCATAAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACGCGGCCGTCTATTACTATGCGAGACCCGGTGGTTT
GGGAGCAGCTCGTCCTTTTGACTATTGGGGGCGAGAGACCACGGTCACGGTTTCTTCG

FAT 64 Heavy Chain Amino Acid sequence SEQ ID NO. 152

Q E Q M Q E S G G E V K K P G S S V G V S
C K A S G G T F S S Y A I S W M R Q A P G
Q G L E W M G W M N P N S G N T G Y A Q K
F Q G R V T M T R N T S I S T A Y M E L S
S L R S E D A A V Y Y Y A R P G G L G A A
R P F D Y W G R E T T V T V S S

FAT 64 Light Chain DNA Sequence SEQ ID NO. 153

CAGTCTGTGCTGACTCAGCCTGCCTCCGTGTCTGGGCCTCCTGGACAGTCAGTCACCATCT
CCTGCACTGGAACCAGCAGTGACATTGGTAGTTATAACTATGTCTCCTGGTACCAACAACA

CCCAGGCGAAGCCCCCAAACTCATGATTTATGAGGTCACTAAGCGGCCCTCAGGGGTCCCT
GATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCGTCTCTGGGCTCCAGG
CTGAGGATGAGGCTGATTATTACTGCAGCTCATATGCAGGCAGCAACACTGTAATTTTCGG
CGGAGGGACCAAGGTCACCGTCCTA

FAT 64 Light Chain Amino Acid sequence SEQ ID NO. 154

Q S V L T Q P A S V S G P P G Q S V T I S
C T G T S S D I G S Y N Y V S W Y Q Q H P
G E A P K L M I Y E V T K R P S G V P D R
F S G S K S G N T A S L T V S G L Q A E D
E A D Y Y C S S Y A G S N T V I F G G G T
K V T V L

FAT 65 Heavy Chain DNA Sequence SEQ ID NO. 155

CAGGTGCAGCTGGTGGAGACCGGGGGAGGCTTGGTCAAGCCTGGAGGGCCCCTGAGACTTT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGCTTCATACATTAGTAGTAGTAGTAGTTACACAAACTACGCA
GACTCTGTGAAGGGCCGATTCACCATTTCCAGAGACAACGCCAAGAACTCACTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGACGCGAGGTG
GTTCGACCCCTGGGGCCAGGGCACCCTGGTCACCGTCTCGAGT

FAT 65 Heavy Chain Amino Acid sequence SEQ ID NO. 156

Q V Q L V E T G G G L V K P G G P L R L S
C A A S G F T F S D Y Y M S W I R Q A P G
K G L E W A S Y I S S S S S S Y T N Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R D A R W F D P
W G Q G T L V T V S S

FAT 65 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 66 Heavy Chain DNA Sequence SEQ ID NO. 157

GAGGTGCAGCTGGTGGAGACTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACCCT
CCTGTGCAGCCTCTGGCTTCATCTTCAGTGACTCTGCTATACACTGGGTCCGCCAGGCTTC
CGGGAAAGGGATGGAGTGGGTCTCATCCATTAGTAGTAGTAGTAGTTACATATACTACGCA
GACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATTTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCCCAGGAGGGAATAGT
AGGGGACGGTATGGACGTCTGGGGCCGAGGGACCACGGTCACCGTCTCCTCT

FAT 66 Heavy Chain Amino Acid sequence SEQ ID NO. 158

E V Q L V E T G G G V V Q P G R S L R P S
C A A S G F I F S D S A I H W V R Q A S G
K G M E W V S S I S S S S S S Y I Y Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A Q E G I V G D G
M D V W G R G T T V T V S S

FAT 66 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 67 Heavy Chain DNA Sequence SEQ ID NO. 159

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGCGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGCTTCTGGTTCCGCCTTTACCAACTACGGTGTCAACTGGGTGCGACAGGCCCC
AGGACAAAGGCTTGAGTGGATGGGATGGATCAGCGCTCACGATGGTGACACAAACTATGCA
CAGAACCTCCGGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGTCTACATGG
ACCTGAGGGGCCTGGAATCTGACGACACGGCCGTATATTACTGTGCGAGTTGTGCGGGGTG
TAGTGGTGGGGATGATGCTTTTGATATCTGGGGCAAGGGAACCCTGGTCACCGTCTCGTCT

FAT 67 Heavy Chain Amino Acid sequence SEQ ID NO. 160

E V Q L V E S G A E A K K P G A S V K V S
C K A S G S A F T N Y G V N W V R Q A P G
Q R L E W M G W I S A H D G D T N Y A Q N
L R G R V T M T T D T S T S T V Y M D L R
G L E S D D T A V Y Y C A S C A G C S G G
D D A F D I W G K G T L V T V S S

FAT 67 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 68 Heavy Chain DNA Sequence SEQ ID NO. 161

CAGGTCCAGCTGGTGCAGTTTGGGGGAGGCTTGGTACAGCCAGGGCGGTCCCTGAGACGCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTAGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGTTGTATCTGC
AAATGGACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGATGCCAGTCGAT
CAGCCATTGGGGCCGAGGCACCCTGGTCACCGTCTCCTCT

FAT 68 Heavy Chain Amino Acid sequence SEQ ID NO. 162

Q V Q L V Q F G G G L V Q P G R S L R R S
C A A S G F T F S S Y A M S B V R Q A P G
K G L E W V S A I S G S G G S T Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M D
S L R A E D T A V Y Y C A R C Q S I S H W
G R G T L V T V S S

FAT 68 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 69 Heavy Chain DNA Sequence SEQ ID NO. 163

CAGGTCATCTTGAGGGAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATCT
CATGTAAAGGGTCTGGATACAGGGTTACCAACTACTGGATTGGCTGGGTGCGCCAGATGCC
CGGGAGAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGC
CCGTCCTTCCAAGGCCAGGTCACCATTTCAGCCGACAAGTCCATCAGCACCGCCTACCTGC
AGTAGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACTGAGTGGCCA
GCTGCTAATGGAGGATGCTTTTGATATCTGGGGCAAAGGGACAATGGTCACCGTCTCTTCA

FAT 69 Heavy Chain Amino Acid sequence SEQ ID NO. 164

Q V I L R E S G A E V K K P G E S L K I S
C K G S G Y R V T N Y W I G W V R Q M P G
R G L E W M G I I Y P G D S D T R Y S P S
F Q G Q V T I S A D K S I S T A Y L Q B S
S L K A S D T A M Y Y C A R L S G Q L L M
E D A F D I W G K G T M V T V S S

FAT 69 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 71 Heavy Chain DNA Sequence SEQ ID NO. 165

CAGGTCACCTTGAAGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGTTACCGGGACCAACCTCAGTGAATTATCCATCCACTGGGCGCGACAGGCTCC
CGGAAAAGGGCTTGAGTGGATGGGAGGTTTTGATCCTGAAGATGTGCAAATAGCCTACGCC
CAGGAGTTCCAGGGCGGACTCGCCATGACCGAGGACACATCCATAGACACAGCCCACATGG
AGCTGAGTAGCCTGAGATCTGAGGACACGGCCGTGTATTTTTGTGTAGCAGGGGGGACTCC
GGTGGTCCACGATGATGCTTTTGAAATTTGGGGCCAGAGGACAATGGTCACCGTGTCTTCA

FAT 71 Heavy Chain Amino Acid sequence SEQ ID NO. 166

Q V T L K E S G A E V K K P G A S V K V S
C K V T G T N L S E L S I H W A R Q A P G
K G L E W M G G F D P E D V Q I A Y A Q E
F Q G G L A M T E D T S I D T A H M E L S
S L R S E D T A V Y F C V A G G T P V V H
D D A F E I W G Q R T M V T V S S

FAT 71 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 72 Heavy Chain DNA Sequence SEQ ID NO. 167

CAGGTACAGCTGCAGCAGTCAGGAGCAGAGGTGAAAAGGCCCGGGGAATCTCTGAAGATCT
CCTGTCAGGGTTCTGGATACAGCTTTCCCAACTCTTGGCTCGCCTGGGTGCGCCAGACGCC
CGGGAAAGACCTGGAGTGGATGGCCATCATCAATCCCGGAAATTCTGATACCAGATACAGC
CCGTCTTTCCAAGGGCAGGTCACCATCACCGCCGACAACTCTATCAGCACCATGTTCTTGC
ACTAGAACAGCCTGAAGGCCTCGGACACCGCCTTGTATTACTGTGCGAGAGCTGGGGTCGC
GGGCGGTGCTTCTGATCTCTGGGGCCAAGGAACTCTGGTCACCGTCTCCAGT

FAT 72 Heavy Chain Amino Acid sequence SEQ ID NO. 168

Q V Q L Q Q S G A E V K R P G E S L K I S
C Q G S G Y S F P N S W L A W V R Q T P G
K D L E W M A I I N P G N S D T R Y S P S
F Q G Q V T I T A D N S I S T M F L H B N
S L K A S D T A L Y Y C A R A G V A G G A
S D L W G Q G T L V T V S S

FAT 72 Light Chain DNA Sequence SEQ ID NO. 169

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCACCTGGTACCAACAGCACCC

AGGCAAAGCCCACAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTCGTAGGT

FAT 72 Light Chain Amino Acid sequence SEQ ID NO. 170

Q S V L T Q P P S V S A A P G Q K V T I S
C S G S T S N I G N N Y V T W Y Q Q H P G
K A H K L M I Y D V S K R P S G V P D R F
S G S K S G N S A S L D I S G L Q S E D E
A D Y Y C A A W D D S L S E F L F G T G T
K L T V V G

FAT 73 Heavy Chain DNA Sequence SEQ ID NO. 171

CAGGTACAGCTGCAGCAGTCAGGAGCAGAGGTGAAGAAGCCCGGGGAGTCTCTGAGGATCT
CCTGTAAGGGTTCAGGATACAACTTTAACACCTATTGGATCGGCTAGGTGCGCCAGGTGCC
CGGGAAAGGCCTGGAGTGGATGGGAATCATCTATCCTCGTGACTCTAATACCAGATATAGC
CCGTCCTTCCAAGGCCAGGTCACCATGTCAGTCGACAAGTACGCCAACACCGCCTACTTAC
AGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGCGCGAAGCATAATATGAT
TGCTCGTCCATATGATCCTTTTGATATCTGGGGCAAGGGCACCCTGGTCACCGTCTCGAGT

FAT 73 Heavy Chain Amino Acid sequence SEQ ID NO. 172

Q V Q L Q Q S G A E V K K P G E S L R I S
C K G S G Y N F N T Y W I G B V R Q V P G
K G L E W M G I I Y P R D S N T R Y S P S
F Q G Q V T M S V D K Y A N T A Y L Q W S
S L K A S D T A M Y Y C A K H N M I A R P
Y D P F D I W G K G T L V T V S S

FAT 73 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 74 Heavy Chain DNA Sequence SEQ ID NO. 173

GGGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATACACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGAATCATCCCTATCTTTGGTACAACATACTACGCA
CAGAATTTCCAGGACAGACTGTCGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AACTGAGCCGCCTGAGATCTGGGGACACGGCCATGTATTACTGTGCGAGAGATGGTCAGGG
GCGTGGCTGGGGACGTGACTGGTATTTCGATATCTGGGGCCGAGGGACAATGGTCACCGTC
TCGA

FAT 74 Heavy Chain Amino Acid sequence SEQ ID NO. 174

G V Q L V Q S G A E V K K P G A S V K V S
C K A S G Y T F T G Y Y I H W V R Q A P G
Q G L E W M G G I I P I F G T T Y Y A Q N
F Q D R L S I T A D E S T S T A Y M E L S
R L R S G D T A M Y Y C A R D G Q G R G W
G R D W Y F D I W G R G T M V T V S

FAT 74 Light Chain DNA Sequence SEQ ID NO. 175

GACATCGTGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCA
TCACTTGCCGGGCCAGTCAGGGTATTAGTAGCTGGTTGGCCTGGTATCAGCAGAAACCAGG
GAGAGCCCCTAAGGTCTTGATCTATAAGGCATCTACTTTAGAAAGTGGGGTCCCATCAAGG
TTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG
ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCGGCCAAGGGAC
CAAGCTGGAGATCAAACGTGCGGCC

FAT 74 Light Chain Amino Acid Sequence SEQ ID NO. 176

D I V M T Q S P S T L S A S V G D R V T I
T C R A S Q G I S S W L A W Y Q Q K P G R
A P K V L I Y K A S T L E S G V P S R F S
G S G S G T D F T L T I S S L Q P E D F A
T Y Y C Q Q S Y S T P W T F G Q G T K L E
I K R A A

FAT 75 Heavy Chain DNA sequence SEQ ID NO. 177

CAGGTGCAGCTACAGCTGTAGGGCGCTGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTCA
CTTGCGCTGTCTATGGTGGGTCCTTCAATGCTGATCACTGGAGCTGGATCCGCCAGCCCCC
AGAGAAGGGGCTAGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGATCTGATATC
CCCGTACTACTACTACGGTATGGACGTCTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCA

FAT 75 Heavy Chain Amino Acid sequence SEQ ID NO. 178

Q V Q L Q L B G A G L L K P S E T L S L T
C A V Y G G S F N A D H W S W I R Q P P E
K G L E W V S A I S G S G G G S T Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A K D L I S P Y Y
Y Y G M D V W G Q G T L V T V S S

FAT 75 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 76 Heavy Chain DNA sequence SEQ ID NO. 179

CAGGTGCAGCTGCAGGAGTCGGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCAGGTATGCTATCAGCTAGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTGACACAAACTATGCA

CAGAACCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGACCACAGCCTACATGG
AGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGGGGGGGGTAT
TCGCGGTATGGACGCCTGGGGGAGAGGACCACGTCACGTCTCGAGT

FAT 76 Heavy Chain Amino Acid sequence SEQ ID NO. 180

Q V Q L Q E S G A E V K K P G S S V K V S
C K A S G G T F S R Y A I S B V R Q A P G
Q G L E W M G W I S A Y N G D T N Y A Q N
L Q G R V T M T T D T S T T T A Y M E L R
S L R S D D T A V Y Y C A R G G G I R G M
D A W G R G P R H V S S

FAT 76 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 77 Heavy Chain DNA Sequence SEQ ID NO. 181

CAGGTGCAGCTGCAGGAGTCCGGGGGAGGCTTGGTACGGCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAGCATAGGCTATGCG
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAGGAACAGGCCGA
CGGTCCGCGTATAGCAGTGGCTGGTACGGGCTACATGGACGTCTGGGGGAGGGGACAATGG
TCACCTGTCTTCAGGTGGAGGAGTTCAA

FAT 77 Heavy Chain Amino Acid sequence SEQ ID NO. 182

Q V Q L Q E S G G G L V R P G R S L R L S
C A A S G F T F D D Y A M H W V R Q A P G
K G L E W V S G I S W N S G S I G Y A D S
V K G R F T I S R D N A K N S L Y L Q M N

S L R A E D T A L Y Y C A K E Q A D G P R
I A V A G T G Y M D V W G R G Q W S P V F
R W R S S

FAT 77 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 78 Heavy Chain DNA Sequence SEQ ID NO. 183

CAGGTGCAGCTGTAGGAGTCGGGGGGAGGCTTGGTGCAGCCTGGAAGGTCTCTGAGACTCT
CCTGTGCAGCCTCTGGATTCAGCTTTGATGACTACGGCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAGCATAGGCTATGCG
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGTGTGAAAGCTGGACGGGG
GGACTACTGGGGCCGGACCACGGTCACCGTCTCCTCA

FAT 78 Heavy Chain Amino Acid sequence SEQ ID NO. 184

Q V Q L B E S G G G L V Q P G R S L R L S
C A A S G F S F D D Y G M H W V R Q A P G
K G L E W V S G I S W N S G S I G Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A L Y Y C V K A G R G D Y W
G R T T V T V S S

FAT 78 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 79 Heavy Chain DNA Sequence SEQ ID NO. 185

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAGCATAGGCTATGCG

GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCCGTATATTACTGTGCGAAAGATCGAAGGAC
ACTCGACTACTTTGACTACTGGGCCGGGGCCAATGTCACCGTGTCTTCA

FAT 79 Heavy Chain Amino Acid sequence SEQ ID NO. 186

Q V Q L V E S G G G L V Q P G R S L R L S
C A A S G F T F D D Y A M H W V R Q A P G
K G L E W V S G I S W N S G S I G Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A K D R R T L D Y
F D Y W A G A N V T V S S

FAT 79 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 82 Heavy Chain DNA Sequence SEQ ID NO. 187

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTTCCTACGACATACACTGGGTCCGCCAAGCTAC
AGGAAAAGGTCTGGAATGGGTCTCAGGTATTGGTACTGCTGGTGACCCATACTATCCAGGC
TCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGAGATCTCCCCCAGTA
TTACTATGACAGTAGTGGATATTACTACCCTGAATACTTCCAGCACTGGGGCCGGGGCACC
CTGGTCACCGTGTCGAGT

FAT 82 Heavy Chain Amino Acid sequence SEQ ID NO. 188

E V Q L V E S G G G V V Q P G G S L R L S
C A A S G F T F S S Y D I H W V R Q A T G
K G L E W V S G I G T A G D P Y Y P G S V
K G R F T I S R D N A K N S L Y L Q M N S

L R A E D T A V Y Y C A R D L P Q Y Y Y D
S S G Y Y Y P E Y F Q H W G R G T L V T V
S S

FAT 82 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 84 Heavy Chain DNA Sequence SEQ ID NO. 189

CAGGTGCAGCTGGTGCAATCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTATTAAATACTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAACCTCAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAAAGGCTATGGGAG
TTCTTACGGGGGAACTTCCTGGGCCCAGGGAACCCTGGTCACACGTTCTTCC

FAT 84 Heavy Chain Amino Acid sequence SEQ ID NO. 190

Q V Q L V Q S G G G V V Q P G R S L R L S
C A A S G F T F S S Y G M H W V R Q A P G
K G L E W V A V I S Y D G S I K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N
N L R A E D T A V Y Y C A K G Y G S S Y G
G T S W A Q G T L V T R S S

FAT 84 Light Chain DNA and Amino Acid sequences

Identical to FAT 7 (SEQ ID NO.'s 25 and 26)

FAT 86 Heavy Chain DNA Sequence SEQ ID NO. 191

CAGGTACAGCTGCAGCAGTCAGGGGGAGGCTTGGTTCAGCCTGGCAGGTCCCTGAGACTCT
CCTGCGTAGTCTCTGGATCTACGTATGTCGGCCCAGCCATACACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAATACGTCGCAGGTATTGGTTGGAGTAGTGATACGAAAGGCTATGCG

GACTCTGTGAGGGGCCAATTCACCATCTCCAGAGACAACGCCAAGAACGCCCTGTATCTGC
AAATGAACAGTCTGAGACCTGAGGACACGGCTGTGTATCACTGTGCGAAGCAATATAGTGG
CTACGATTATTGGGACTACTTTGACTACTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

FAT 86 Heavy Chain Amino Acid sequence SEQ ID NO. 192

Q V Q L Q Q S G G G L V Q P G R S L R L S
C V V S G S T Y V G P A I H W V R Q A P G
K G L E Y V A G I G W S S D T K G Y A D S
V R G Q F T I S R D N A K N A L Y L Q M N
S L R P E D T A V Y H C A K Q Y S G Y D Y
W D Y F D Y W G Q G T T V T V S S

FAT 86 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 87 Heavy Chain DNA Sequence SEQ ID NO. 193

CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGAAGTCTCTGGATTGAGGTTCAGCAGCTACGGCATGAATTGGGTCCGCCAGGCTCC
AGGGAAGGCACTGGAGTGGGTCTCATCCATAGCAACCACTGAAAGATTCACATCGTACGCA
GACTCAGTGAAGGGCCGATTCTCCATCTCCAGAGACGACGCCAAGAACTCAGTTTATCTGC
AGATGGACAGCCTGAGGGCCGAGGACACGGCCGTATATTACTGTGCGAAGTCGAAGGTAGG
GGGTGGCAATGACTACTGGGGCAGAGGGACAATGGTCACCGTCTCCTCA

FAT 87 Heavy Chain Amino Acid sequence SEQ ID NO. 194

Q V Q L V Q S G G G L V K P G G S L R L S
C E V S G L R F S S Y G M N W V R Q A P G
K A L E W V S S I A T T E R F T S Y A D S
V K G R F S I S R D D A K N S V Y L Q M D

S L R A E D T A V Y Y C A K S K V G G G N
D Y W G R G T M V T V S S

FAT 87 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 88 Heavy Chain DNA Sequence SEQ ID NO. 195

GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTTAGCAGATATGCTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTTCCTATGGTACAGCAAACTACGCA
CAGAGGTTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGG
AGCCGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATTATAGCAG
CAGACGGTACAGCTACTTTGACTACTAGGGCCAGGGAACCTTGGTCACAGTGTCCTCA

FAT 88 Heavy Chain Amino Acid sequence SEQ ID NO. 196

E V Q L V Q S G A E V K K P G A S V K V S
C K A S G G T F S R Y A I S W V R Q A P G
Q G L E W M G G I I P S Y G T A N Y A Q R
F Q G R V T M T T D T S T S T A Y M E P R
S L R S D D T A V Y Y C A R D Y S S R R Y
S Y F D Y B G Q G T L V T V S S

FAT 88 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 89 Heavy Chain DNA Sequence SEQ ID NO. 197

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGGGACCCTGTCCCTCA
CCTGCGCTGTGTCTGGTGCCTCCATCAGCAATGGTTTCTGGTGGGGCTGGGTACGCCAGCC
CCCAGGGAGGGGTCTGGAGTGGATTGGGGAAATCTTTTTTACTGGACGCGCCAATTACAAC
CCGTCCCTGAAGAGTCGAGTCACCACATCAGTAGACGAGTCCAAGAACCAGTTCTCCCTGA

AGTTGACCTCTGTGACCGCCGCGGACACGGCCGTATATTTCTGTGCGAGAGACCGGGACAC
TGGCTAGTACTTCTTTGACGACTGGGGCAAAGGGACAATGGTCACCGTCTCGAGT

FAT 89 Heavy Chain Amino Acid sequence SEQ ID NO. 198

Q V Q L Q E S G P G L V K P S G T L S L T
C A V S G A S I S N G F W W G W V R Q P P
G R G L E W I G E I F F T G R A N Y N P S
L K S R V T T S V D E S K N Q F S L K L T
S V T A A D T A V Y F C A R D R D T G B Y
F F D D W G K G T M V T V S S

FAT 89 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 90 Heavy Chain DNA sequence SEQ ID NO. 199

GAGGTGCAGCTGGTGGAGACCGGGGGAGGCTTGGTACAGCCTGGCAGGTCCGTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGCTCCGGCAACCTCC
AGGGAAGGGCCTGGAGTAGGTCTCAACTATTAGTGGTAGTGGTGGTAGCACGTACTACGCA
GACTCCGTGAAGGGCCGGATCACCATCTCCAGAGACAATTCCAAGAACACGCCGTATCTGC
AAATGAACAGCCTGAGAGTCGGGGACACGGCCGCATATTACTGTGCGAAAGACCCCTATTG
TGGTAGTGCCAGCTGCTATACTTATCATGCTTTTGATCTCTGGGGCCAAGGCACCCTGGTC
ACCGTCTCGAGT

FAT 90 Heavy Chain Amino Acid sequence SEQ ID NO. 200

E V Q L V E T G G G L V Q P G R S V R L S
C A A S G F T F D D Y A M H W L R Q P P G
K G L E B V S T I S G S G G S T Y Y A D S
V K G R I T I S R D N S K N T P Y L Q M N
S L R V G D T A A Y Y C A K D P Y C G S A
S C Y T Y H A F D L W G Q G T L V T V S S

FAT 90 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 91 Heavy Chain DNA Sequence SEQ ID NO. 201

CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCTTGGTACAGCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCAGGTTTGATGATTATGCCATGCACTAGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCGCTGGTATTGATTGGAATAGTGGTTCCATCGGCTATGTG
GACTCTGTGAAGGGCCGATTCACCCTCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGACAAAGAGTA
TAGCAGCTCGTACTACTTTGACTACTGGGGCCGGGGACAATGGTTCACAGTTCTGCTTC

FAT 91 Heavy Chain Amino Acid sequence SEQ ID NO. 202

Q V Q L Q E S G G G L V Q P G R S L R L S
C A A S G F R F D D Y A M H B V R Q A P G
K G L E W V A G I D W N S G S I G Y V D S
V K G R F T L S R D N A K N S L Y L Q M N
S L R A E D T A L Y Y C A K D K E Y S S S
Y Y F D Y W G R G Q W F T V L L

FAT 91 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 92 Heavy Chain DNA sequence SEQ ID NO. 203

GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCGTTGCGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTACTCATGGCATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCCGGAGTGGCTGACATTTATCTCATATGATGAGAGTGAAAAATCTTATGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCGAGAAAACACTGTATCTGC
AAATGAACAGTCTGAGACCTGAGGACACGGCTGTGTATTACTGTGCGAAAGATGTCTTGAT
ACACCAAACGTACAAGTGGTTCGACCCCTGGGGCAAGGGCACCCTGGTCACCGTCTCCTCA

FAT 92 Heavy Chain Amino Acid sequence SEQ ID NO. 204

E V Q L V Q S G G G V V Q P G R S L R L S
C A A S G F T F S T H G M H W V R Q A P G
K G P E W L T F I S Y D E S E K S Y A D S
V K G R F T I S R D N S E K T L Y L Q M N
S L R P E D T A V Y Y C A K D V L I H Q T
Y K W F D P W G K G T L V T V S S

FAT 92 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 93 Heavy Chain DNA sequence SEQ ID NO. 205

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGAGGGTCCCTGAGACTCC
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTATGAAATGAACTGGGTCCGCCAGGCTCC
AGGGAGGGGGCGGGAGTGGGTCTCGGGTATTAATTGGAATGGTGGTAACACAGGTTATGCG
GACTCTGTGAAGGGCCGATTTACCATCTCCCGAGACAACGCCAGGAACTCCCTGTATCTGC
AAATGAACAGTCCGAGAGCCGAGGACACGGCCTTGTATTCCTGTGTGAGAGATCGGAATCA
ATACTATGATAGTGGTGGTTATCCTGATTCTTTTGATATCTGGGGCCAGTGGACAATGGTC
ACAGTCTCTTCA

FAT 93 Heavy Chain Amino Acid sequence SEQ ID NO. 206

Q V Q L V E S G G G L V Q P G G S L R L P
C A A S G F T F S S Y E M N W V R Q A P G
R G R E W V S G I N W N G G N T G Y A D S
V K G R F T I S R D N A R N S L Y L Q M N
S P R A E D T A L Y S C V R D R N Q Y Y D
S G G Y P D S F D I W G Q W T M V T V S S

FAT 93 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 94 Heavy Chain DNA sequence SEQ ID NO. 207

GAGGTGCAGCTGGTGGAGACCGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTGCATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTCCATACATTAGTAGTAGTAGTAGTACCATATACTACGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTATCTAC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGACTTGGTACGGA
GACTATTGACTATTGGGGCGGGGACCACGTCACCGTCTCGAGTT

FAT 94 Heavy Chain Amino Acid sequence SEQ ID NO. 208

E V Q L V E T G G G L V K P G G S L R L S
C A A S G F T F S D Y C M S W I R Q A P G
K G L E W V P Y I S S S S S S T I Y Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R L G T E T I D
Y W G G D H V T V S S

FAT 94 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 95 Heavy Chain DNA Sequence SEQ ID NO. 209

CAGGTCACCTTGAAGGAGTCTGGGGGAGGCTTGGTACAGCCCGGCAGGCCCCTGAGACTCT
CCTGTGCGGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAGCATAGGCTATGCG
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGTGGAAAAGATTTGAGTGC
GGGCGGTATGGACGTCTGGGGGCAAGGGACCACGGTCACCGTCTCCTCA

FAT 95 Heavy Chain Amino Acid sequence SEQ ID NO. 210

Q V T L K E S G G G L V Q P G R P L R L S
C A A S G F T F D D Y A M H W V R Q A P G
K G L E W V S G I S W N S G S I G Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A L Y Y C G K D L S A G G M
D V W G Q G T T V T V S S

FAT 95 Light Chain DNA Sequence SEQ ID NO. 211

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAACA
CCCAGGCAAAGCCCCCAAACTCATGATTTATGAGGGCAGTAAGCGGCCCTTAGGGGTCCCT
GACCGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGT
CTGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCCT
CGGAACTGGGACCAAGCTGACCGTCCTA

FAT 95 Light Chain Amino Acid sequence SEQ ID NO. 212

Q S V L T Q P P S V S A A P G Q S I T I S
C T G T S S D V G G Y N Y V S W Y Q Q H P
G K A P K L M I Y E G S K R P L G V P D R
F S G S K S G N S A S L D I S G L Q S E D
E A D Y Y C A A W D D S L S E F L L G T G
T K L T V L

FAT 96 Heavy Chain DNA Sequence SEQ ID NO. 213

GAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGGCTTCCGGTTACATGTTTACCAGTCACGGTATCACCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGGTGACAATGTTAGCACAAACTATGCA
GAGAAGCTTCTGGGCAGAGTCACCATGACCACAGACACATCCACGAGTACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGTACAGGGTCCCT
ATTTGACTACTGGGGCCAGGCACCCCGGTCACCGTCTCCTCA

FAT 96 Heavy Chain Amino Acid sequence SEQ ID NO. 214

E V Q L V E S G A E V K K P G A S V K V S
C K A S G Y M F T S H G I T W V R Q A P G
Q G L E W M G W I S G D N V S T N Y A E K
L L G R V T M T T D T S T S T A Y M E L S
S L R S E D T A V Y Y C A S T G S L F D Y
W G R G T P V T V S S

FAT 96 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 97 Heavy Chain DNA Sequence SEQ ID NO. 215

GAAGTGCAGCTGGTGCAGTCTGGGGCTGAGATGAAGAAGCCTGGGTCCTCGGTGAAAGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCACCTATATTATCAACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATGTTTGATACAACAAACTACGCA
CAGAAGTTCCAGGGCAGAGTCTCCATTACCGCGGACGAATCCACGAGGACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGACGACACGGCCCTCTATTACTGTGCGAGAGATCCGTTGGG
GACCACAGGAGCTTTTGATATCTGGGGCAGAGGCACCCTGGTCACAGTCTCGAGT

FAT 97 Heavy Chain Amino Acid sequence SEQ ID NO. 216

E V Q L V Q S G A E M K K P G S S V K V S
C K A S G G T F S T Y I I N W V R Q A P G
Q G L E W M G G I I P M F D T T N Y A Q K
F Q G R V S I T A D E S T R T A Y M E L S
S L R S D D T A L Y Y C A R D P L G T T G
A F D I W G R G T L V T V S S

FAT 97 Light Chain DNA Sequence SEQ ID NO. 217

CAGTCTGTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCCCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAACA
CCCAGGCAAAGCCCCCAAACTCATGATTTATGAGGGCAGTAAGCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAATACGGCCTCCCTGACAATCTCTGGGCTCCAGG
CTGAGGATGAGGCTGATTATTACTGCAGTTCATATGCAGGCATCAACAATTTCGGGGTGCT
ATTCGGCGGAGGGACCAAGCTGACCGTCCTA

FAT 97 Light Chain Amino Acid sequence SEQ ID NO. 218

Q	S	V	L	T	Q	P	A	S	V	S	G	S	P	G	Q	S	I	T	I	S
C	T	G	T	S	S	D	V	G	G	Y	N	Y	V	S	W	Y	Q	Q	H	P
G	K	A	P	K	L	M	I	Y	E	G	S	K	R	P	S	G	V	S	N	R
F	S	G	S	K	S	G	N	T	A	S	L	T	I	S	G	L	Q	A	E	D
E	A	D	Y	Y	C	S	S	Y	A	G	I	N	N	F	G	V	L	F	G	G
G	T	K	L	T	V	L														

FAT 98 Heavy Chain DNA Sequence SEQ ID NO. 219

GAAGTGCAACTGGTGCAGTCTGGCGGAGGGTTGGTTTGGCCTGGGGGGTCCCTGAGACTCT
CCTGTGAGGCTTCTGGATTCTTAGTTTCAGCTAGTGGACAGATGAACTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGAGTGGGTCTCATTTATTAGTAGTGGTAGTAGTACCACATACTAC
GCAGACTCTGTGAGGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACACTGTATC
CGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGAGAGGCCGA
CTACTACTACGGTATGGACGTCTGGGGGCGAGGCACCCTTGTCACAGTGTCGAGT

FAT 98 Heavy Chain Amino Acid sequence SEQ ID NO. 220

E	V	Q	L	V	Q	S	G	G	G	L	V	W	P	G	G	S	L	R	L	S
C	E	A	S	G	F	L	V	S	A	S	G	Q	M	N	W	V	R	Q	A	P
G	K	G	L	E	W	V	S	F	I	S	S	G	S	S	T	T	Y	Y	A	D
S	V	R	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	P	Q	M

N S L R A E D T A V Y Y C A R E A D Y Y Y
G M D V W G R G T L V T V S S

FAT 98 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 99 Heavy Chain DNA Sequence SEQ ID NO. 221

CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTACAGCCTAGGGGGCCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGGTATTAGTGGTAGTGGTGGTTCCACATACTACGCA
GACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATGGAGAAGG
GACTACTGGGGCCGAGGGACAATGGTCCACAGTCTCGAGT

FAT 99 Heavy Chain Amino Acid sequence SEQ ID NO. 222

Q V Q L V Q S G G G L V Q P R G P L R L S
C A A S G F T F S S Y A M S W V R Q A P G
K G L E W V S G I S G S G G G S T Y Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R D G E G T T G
A E G Q W S T V S S

FAT 99 Light Chain DNA Sequence SEQ ID NO. 223

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGACTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC
AGGCAAAGCCCTCAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTCCTA

FAT 99 Light Chain Amino Acid sequence SEQ ID NO. 224

Q S V L T Q P P S V T A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A L K L M I Y D V S K R P S G V P D R F
S G S K S G N S A S L D I S G L Q S E D E
A D Y Y C A A W D D S L S E F L F G T G T
K L T V L

FAT 101 Heavy Chain DNA Sequence SEQ ID NO. 225

GAGGTGCAGCTGGTGGAGTCCGGAGGGGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCAGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCGCATACTACGCA
GACTCCGTGAAGGGCCGGTTCACCATTCCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAAAGCCTATGGCAG
TGAAGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

FAT 101 Heavy Chain Amino Acid sequence SEQ ID NO. 226

E V Q L V E S G G G L V Q P G G S L R L S
C A A S G F T F S S Y A M S W V R Q A P G
K G Q E W V S A I S G S G G G S A Y Y A D S
V K G R F T I P R D N S K N T L Y L Q M N
S L R A E D T A V Y Y C A K A Y G S E D Y
W G Q G T L V T V S S

FAT 101 Light Chain DNA and Amino Acid sequences

Identical to FAT 44 (SEQ ID NO.'s 115 and 116)

FAT 102 Heavy Chain DNA Sequence SEQ ID NO. 227

CAGGTCACCTTGAAGGAGTCTGGGGGAGGCGTGGTCCAACCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGAATTCAGCCTCAGTAGCCATGCTATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCCAACATAAAGGGAGATGGAAGTGCGAAGTACTCTGTG
GACTCTATGAAGGGCCGATTCACCATCCCCAGAGACAACGCCAAAGACTCAGTGTATCTGG

AAATGACCAGCCTGAGAGCCGAGGACACGGCCGTGTACTACTGTGCGAGAGACCTGAACCC
GGGCCAGGGGGGGACATATTATGATGCTTTTGACATTTGGGGGCAAGGCACCCCGGTCACC
GTCTCCTCA

FAT 102 Heavy Chain Amino Acid sequence SEQ ID NO. 228

Q	V	T	L	K	E	S	G	G	G	V	V	Q	P	G	R	S	L	R	L	S
C	A	A	S	E	F	S	L	S	S	H	A	M	H	W	V	R	Q	A	P	G
K	G	L	E	W	V	A	N	I	K	G	D	G	S	A	K	Y	S	V	D	S
M	K	G	R	F	T	I	P	R	D	N	A	K	D	S	V	Y	L	E	M	T
S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	D	L	N	P	G	Q	G
G	T	Y	Y	D	A	F	D	I	W	G	Q	G	T	P	V	T	V	S	S	

FAT 102 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 103 Heavy Chain DNA Sequence SEQ ID NO. 229

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAAACTCT
CCTGTGCAGCCTCTGGGTTCACCTTCAGTGGCTCTGCTATGCACTGGGTCCGCCAGGCTTC
CGGGAAAGGGCTGGAGTGGGTTGGCCGTATTAGAAGCAAAGCTAACAGTTACGCGACAGCA
TATGCTGCGTCGGTGAAAGGCAGGTTCACCATCTCCAGAGATGATTCAAAGAACACGGCGT
ATCTGCAAATGAACAGCCTGAAAACCGAGGACACGGCCGTGTATTACTATACTAGACCTGG
AGATAGCAGTGGCGGTATGGGGAGGGACTACTGGGGCAGGGGCACCCTGGTCACCGTCTCG
AGT

FAT 103 Heavy Chain Amino Acid sequence SEQ ID NO. 230

E	V	Q	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	L	K	L	S
C	A	A	S	G	F	T	F	S	G	S	A	M	H	W	V	R	Q	A	S	G
K	G	L	E	W	V	G	R	I	R	S	K	A	N	S	Y	A	T	A	Y	A

A S V K G R F T I S R D D S K N T A Y L Q
M N S L K T E D T A V Y Y Y T R P G D S S
G G M G R D Y W G R G T L V T V S S

FAT 103 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 104 Heavy Chain DNA Sequence SEQ ID NO. 231

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGTTTGGTACAGCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTAGTAGTTACACAAACTACGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGATCGGCGTA
TTACGATATTTTGACTGGCTCGGGGGATGATGCTTTTGATATCTGGGGCCGAGACACCCTG
GTCACCGTCTCGAGT

FAT 104 Heavy Chain Amino Acid sequence SEQ ID NO. 232

E V Q L V E S G G G L V Q P G R S L R L S
C A A S G F T F D D Y A M H W V R Q A P G
K G L E W V S Y I S S S S S Y T N Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R G S A Y Y D I
L T G S G D D A F D I W G R D T L V T V S
S

FAT 104 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 105 Heavy Chain DNA sequence SEQ ID NO. 233

GGGGTGCAGCTGGTCCATTCTGGGGCTGAGGTGAAGAAGCCTGGTTCTTCGGTGAAGTTCT
CCTGCAAGGCTTCTGGAGACACTTTCAACACTTATGTTATCAACTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATGTTTGGAACAGCAAGCCACGCA
CAGAAGTTTCAGGGCAGAGTCACACTTACCGCGGACGAATCTATTAACACAGTGTACATGG
AGCTGAGCGGGCTCAGGTATGACGACGCGGCCGTATATTATTGTGCGCGAGAAGTTATATT
CTTCTCCGAAGGCATGGACGTCTGGGGCAGAGGAACCCTGGTCACCGTCTCGAGT

FAT 105 Heavy Chain Amino Acid sequence SEQ ID NO. 234

G	V	Q	L	V	H	S	G	A	E	V	K	K	P	G	S	S	V	K	F	S
C	K	A	S	G	D	T	F	N	T	Y	V	I	N	W	V	R	Q	A	P	G
Q	G	L	E	W	M	G	G	I	I	P	M	F	G	T	A	S	H	A	Q	K
F	Q	G	R	V	T	L	T	A	D	E	S	I	N	T	V	Y	M	E	L	S
G	L	R	Y	D	D	A	A	V	Y	Y	C	A	R	E	V	I	F	F	S	E
G	M	D	V	W	G	R	G	T	L	V	T	V	S	S						

FAT 105 Light Chain DNA sequence SEQ ID NO. 235

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGCCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCGGGAGTAACACTGTAAACTGGTACCAGCGACTCCC
AGGAGCGGCCCCCCAACTCCTCATCTACAATAATGACCAGCGGCCCTCAGGGATCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGGCTCCCTGGTCATCAGTGGGCTCCAGTCTG
AAGATGAGGCTGATTACTACTGTGCGTCATGGGATGACAGTCTGAATGGTCGGGTGTTCGG
CGGAGGGACCAAGCTGACCGTCCTA

FAT 105 Light Chain Amino Acid sequence SEQ ID NO. 236

Q	S	V	L	T	Q	P	P	S	A	S	G	T	P	G	Q	R	A	T	I	S
C	S	G	S	S	S	N	I	G	S	N	T	V	N	W	Y	Q	R	L	P	G
A	A	P	Q	L	L	I	Y	N	N	D	Q	R	P	S	G	I	P	D	R	F
S	G	S	K	S	G	T	S	G	S	L	V	I	S	G	L	Q	S	E	D	E
A	D	Y	Y	C	A	S	W	D	D	S	L	N	G	R	V	F	G	G	G	T
K	L	T	V	L																

FAT 106 Heavy Chain DNA sequence SEQ ID NO. 237

CAGGTGCAGCTGCAGGAGTCGGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGCTGAAGGTTT
CGTGCCAGGCTTCTGCAGGCACCTTCAACAGCCATGCTATCGGCTGGGTGCGGCAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGATCAATCCTAACAGTGGTGGCACAAACTATGCA
GAGAAATTTCAGGGCAGGGTCACCCTGACCAGGGACGCGGCCATCAGTACAGCGTACTTGG
AGCTGAGCAGGCTGACATCTGACGACACGGCCATGTATTACTGTGCGAGAGATATCGATGA
TAGTGGTTATCAATACTGGGGCCAGAGCACCCTGGTCACCGTCTCTTCC

FAT 106 Heavy Chain Amino Acid sequence SEQ ID NO. 238

Q V Q L Q E S G A E V K K P G S S L K V S
C Q A S A G T F N S H A I G W V R Q A P G
Q G L E W M G W I N P N S G G T N Y A E K
F Q G R V T L T R D A A I S T A Y L E L S
R L T S D D T A M Y Y C A R D I D D S G Y
Q Y W G Q S T L V T V S S

FAT 106 Light Chain DNA sequence SEQ ID NO. 239

CAGTCTGTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACTCATGATTTATGAGGTCAATAAGCGCCCCTCAGGGGTCCCT
GATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCGTCTCTAGACTCCAGG
CTGAGGATGAGGCTGATTATTACTGCAGCTCATATGCAGGCAACGACAGTGTGCTTTTCGG
CGGAGGGACCAAGCTGACCGTCCTA

FAT 106 Light Chain Amino Acid sequence SEQ ID NO. 240

Q S V L T Q P A S V S G S P G Q S I T I S
C T G T S S D V G G Y N Y V S W Y Q Q H P
G K A P K L M I Y E V N K R P S G V P D R
F S G S K S G N T A S L T V S R L Q A E D
E A D Y Y C S S Y A G N D S V L F G G G T
K L T V L

FAT 107 Heavy Chain DNA Sequence SEQ ID NO. 241

CAGGTACAGCTGCAGCAGTCAGGCCCAGGGCTGGTGAAGCCTTCGGGGACCCTGTCCCTCA
CCTGCGGTGTCTCTGGTGACTCCATGAGTGGTAATAACCGGTGGAGTTGGGTCCGCCAGTC
CCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTAC
GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATT
TGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGGCAGACACATA
TAGTGGCTACGATGAGGCCCCCACAAACTGGGGCCGAGGCACCCTGGTCACGGTATCGAGT

FAT 107 Heavy Chain Amino Acid sequence SEQ ID NO. 242

Q	V	Q	L	Q	Q	S	G	P	G	L	V	K	P	S	G	T	L	S	L	T
C	G	V	S	G	D	S	M	S	G	N	N	R	W	S	W	V	R	Q	S	P
G	K	G	L	E	W	V	S	A	I	S	G	S	G	G	S	T	Y	Y	A	D
S	V	K	G	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M
N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	A	D	T	Y	S	G	Y
D	E	A	P	T	N	W	G	R	G	T	L	V	T	V	S	S				

FAT 107 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 108 Heavy Chain DNA Sequence SEQ ID NO. 243

CAGGTGCAGCTGGTGCAGTTTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCAAGACTTCTGGATACAGCTTCACAAATTATGATATCAACTGGGTGCGACAGGCCGC
TGGGCAAGGGCTTGAGTGGATGGGATGGATCAGCGCTCACAATGGTGACACAAACTACGCA
CAGAAGGTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGACCATAGGCTACATGG
AGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGCCTTCAACCT
GGGCGACAGTGACTACGAGTTGGAGGGTGATGCTTTTGATATCTGGGGCCAAGGGACAATG
GTCACCGTATCGTCA

FAT 108 Heavy Chain Amino Acid sequence SEQ ID NO. 244

Q V Q L V Q F G A E V K K P G A S V K V S
C K T S G Y S F T N Y D I N W V R Q A A G
Q G L E W M G W I S A H N G D T N Y A Q K
V Q G R V T M T T D T S T T I G Y M E L R
S L R S D D T A V Y Y C A R A F N L G D S
D Y E L E G D A F D I W G Q G T M V T V S
S

FAT 108 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 109 Heavy Chain DNA Sequence SEQ ID NO. 245

CAGGTGCAGCTGGTGCAGTTTGGGGGAGGCTTGGTTCAGCCTGGCAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGGCAAGCTCC
AGGGAAGGGCCTGGAGTGGGTCTCAGGTATTAGTTGGAATAGTGGTAGCATAGGCAATGCG
GACTCTGTGAAGGGCCGATTCACCATTTCCAGAGACAACGCCAAGAACTCCCTGTATCTGC
AAATGAACAGTTTGAGAGCTGAGGACACGGCCTTGTATTACTGTGCAAAAGATATTTCCAA
CATTGTATTAGCACCAGCTGCCACTACATCCCACTTTGACTACTGGGGGAGGGGGACCACG
GTCACCGTATCGAC

FAT 109 Heavy Chain Amino Acid sequence SEQ ID NO. 246

Q V Q L V Q F G G G L V Q P G R S L R L S
C A A S G F T F D D Y A M H W V R Q A P G
K G L E W V S G I S W N S G S I G N A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A L Y Y C A K D I S N I V L
A P A A T T S H F D Y W G R G T T V T V S

FAT 109 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 110 Heavy Chain DNA Sequence SEQ ID NO. 247

GAGGTGCAGCTGGTGGAGTCTGGAGCTGAGGTGAAGCAGCCTGGGGCCTCAGTGAAGGTCT
CCTGCCAGGCCTCTGGTTACTCCTTTAGCAGTCATGGTATCAGCTGGGTGCGACAGGCCCC
AGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAAGGGAAACACAAACTATACA
CAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACCCATCGACGAGCACAGCCTACATGG
AACTGAGGAGCCTGAGACCTGACGACACGGCCATGTATTACTGTGCGAGCCAATATGACAT
TATGACTGCTTATCACACTCATGGAATGGACGTCTGGGGGCGGGGGACCACGGTCACCGTC
TCGAGT

FAT 110 Heavy Chain Amino Acid sequence SEQ ID NO. 248

E	V	Q	L	V	E	S	G	A	E	V	K	Q	P	G	A	S	V	K	V	S
C	Q	A	S	G	Y	S	F	S	S	H	G	I	S	W	V	R	Q	A	P	G
Q	G	L	E	W	M	G	W	I	S	A	Y	K	G	N	T	N	Y	T	Q	K
L	Q	G	R	V	T	M	T	T	D	P	S	T	S	T	A	Y	M	E	L	R
S	L	R	P	D	D	T	A	M	Y	Y	C	A	S	Q	Y	D	I	M	T	A
Y	H	T	H	G	M	D	V	W	G	R	G	T	T	V	T	V	S	S		

FAT 110 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 111 Heavy Chain DNA Sequence SEQ ID NO. 249

GAGGTGCAGCTGATGGAGTCGGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCT
CCTGCAAGGCTTCTGGAGGCACCTTCAGCAACTCTCCTATCAACTGGCTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGAAGTATCATCCCTTCCTTTGGTACAGCAAACTACGCT
CAGAAGTTCCAGGGCAGACTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACATGG
AGCTGAGCAGCCTGAGATCCGAGGACACGGCCGTGTATTACTGTGCGGCAGATAGTGGCTA
CGATTCTCCGTTCTACTGGGGGAAGGGGACCACGGTCACCGTCTCGAGT

FAT 111 Heavy Chain Amino Acid sequence SEQ ID NO. 250

E V Q L M E S G A E V K K P G S S V K V S
C K A S G G T F S N S P I N W L R Q A P G
Q G L E W M G S I I P S F G T A N Y A Q K
F Q G R L T I T A D E S T S T A Y M E L S
S L R S E D T A V Y Y C A A D S G Y D S P
F Y W G K G T T V T V S S

FAT 111 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 112 Heavy Chain DNA Sequence SEQ ID NO. 251

GAGGTCACCTTGAAGGAGTCTGGGGGAGGCTTAGTCAAGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTAATTTCACCTTCAGTGACTTCTACATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTATCAGAGGTACTTACACAAAGTAC
GCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTATATCACTGTGCGAGAGATTTTGA
CTCCGGTGGTAACTCCGCCATTTTTGATATCTGGGCAAAGGACCACGTCACCGTCTCCTCC

FAT 112 Heavy Chain Amino Acid sequence SEQ ID NO. 252

E V T L K E S G G G L V K P G G S L R L S
C A A S N F T F S D F Y M S W I R Q A P G
K G L E W V S Y I S S I R G T Y T K Y A D
S V K G R F T I S R D N A K N S L Y L Q M
N S L R A E D T A V Y H C A R D F D S G G
N S A I F D I W A K D H V T V S S

FAT 112 Light Chain DNA Sequence SEQ ID NO. 253

CAGTCTGTGTTGACGCAGCCTCCCTCAGTGTATGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC

AGGCAAAGCCCCCAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTTTCTAAT
CGCTTCTCTGGCTCCAAGTCTGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTCCTA

FAT 112 Light Chain Amino Acid sequence SEQ ID NO. 254

Q S V L T Q P P S V Y A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A P K L M I Y D V S K R P S G V S N R F
S G S K S G N S A S L D I S G L Q S E D E
A D Y Y C A A W D D S L S E F L F G T G T
K L T V L

FAT 113 Heavy Chain DNA Sequence SEQ ID NO. 255

CAGGTGCAGCTGGTGGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGCGAAGGTCT
CTTGCAAGGCTTGTGGAGGCACCTTCAGCAGATATGCTATCAACTGGGTGCGACAGGCCCC
TGGGCAAGGGCTTGAGTGGATGGGAGCAATCCTCCCTGTCTTTGGTACAACAAACTACGCT
CAGAAGCTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTATATGG
AGCTGAGAAGGCTGACATCTGAGGACACGGCCGTGTATTACTGTGCAACATGTGCGGAATT
TTGTAGTGATTCCAACTGCCCTCTAGACCCCTGGGGCAAAGGGACAGTGGTCACCGTCTCC
TCCA

FAT 113 Heavy Chain Amino Acid sequence SEQ ID NO. 256

Q V Q L V E S G A E V K K P G S S A K V S
C K A C G G T F S R Y A I N W V R Q A P G
Q G L E W M G A I L P V F G T T N Y A Q K
L Q G R V T M T E D T S T D T A Y M E L R
R L T S E D T A V Y Y C A T C A E F C S D
S N C P L D P W G K G T V V T V S S

FAT 113 Light Chain DNA Sequence SEQ ID NO. 257

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTT
CCTGCTCTGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCC
AGGCAAAGCCCCCAAACTCATGATTTATGATGTCAGTAAGCGGCCCTCAGAGGTCCCTGAC
CGATTCTCTGGCTCCAAGTATGGCAACTCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGAATTTCTCTTCGG
AACTGGGACCAAGCTGACCGTCCTA

FAT 113 Light Chain Amino Acid sequence SEQ ID NO. 258

Q S V L T Q P P S V S A A P G Q K V T I S
C S G S T S N I G N N Y V S W Y Q Q H P G
K A P K L M I Y D V S K R P S E V P D R F
S G S K Y G N S A S L D I S G L Q S E D E
A D Y Y C A A W D D S L S E F L F G T G T
K L T V L

FAT 114 Heavy Chain DNA Sequence SEQ ID NO. 259

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCTTTCGGGGAATCTGTCCCTCA
CCTGCGCTGTCTCTGGTGTGTCCCTCAGCAGTAGTGACTGGTGGTGGCTCCACCAGGCTCC
AGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTACGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAAAGATATAGCAGA
GGGCGTAGGCTACTACTACATGAACGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCA

FAT 114 Heavy Chain Amino Acid sequence SEQ ID NO. 260

Q V Q L Q E S G P G L V K L S G N L S L T
C A V S G V S L S S S D W W W L H Q A P G
K G L E W V A V I S Y D G S N K Y Y A D S
V K G R F T I S R D N S K N T L Y L Q M N

S L R A E D T A V Y Y C A K D I A E G V G
Y Y Y M N V W G Q G T M V T V S S

FAT 114 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 115 Heavy Chain DNA Sequence SEQ ID NO. 261

GGGGTGCAGCTGGTGGAGTCCGGGGGAGCCTTGGCACAGCCTGGGGGGTCCCTAAGACTTT
CTTGTGCAGCCTCTGGATTCACGTTTAGCAGCTATGCCATGATCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCTTCTATCAGTGGTAGTGGTGGTGGCACATATTACGCA
GACTCCGTGAAGGGCCGGTTCACCGTCTCCAGAGACAATTCCAAGAACACGGTGTATCTGC
AGATGAACAGTCTGAGAGCCGGGGACACGGCCGTTTATTATTGTGCGAAGAGGGCCAACTA
CTACTACTTGGACGTCTGGGGCCGAGGAACCATTGTGCCGTGG

FAT 115 Heavy Chain Amino Acid sequence SEQ ID NO. 262

G V Q L V E S G G A L A Q P G G S L R L S
C A A S G F T F S S Y A M I W V R Q A P G
K G L E W V S S I S G S G G G T Y Y A D S
V K G R F T V S R D N S K N T V Y L Q M N
S L R A G D T A V Y Y C A K R A N Y Y Y L
D V W G R G T I V P W

FAT 115 Light Chain DNA Sequence SEQ ID NO. 263

TCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCA
CATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACA
GGCCCCTGTACTTGTCATCTATGGTAAAAATAAGCGGCCCTCAGGGATCCCAGACCGACTC
TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAGGATG
AGGCTGACTATTACTGTAACTCCCGGGACATCAGTGGTAACCATGTGCTTTTCGGCGGAGG
GACCAAGCTGACCGTCCTA

FAT 115 Light Chain Amino Acid sequence SEQ ID NO. 264

S S E L T Q D P A V S V A L G Q T V R I T
C Q G D S L R S Y Y A S W Y Q Q K P G Q A
P V L V I Y G K N K R P S G I P D R L S G
S S S G N T A S L T I T G A Q A E D E A D
Y Y C N S R D I S G N H V L F G G G T K L
T V L

FAT 116 Heavy Chain DNA Sequence SEQ ID NO. 265

GAGGTGCAGCTGGTGGAGTTTGGGGGAGGCTTGGTCAAGCCTGGAGAGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTAGTAGTTACACAAACTACGCA
GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATTTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCGGGACCCA
GTGCTCATTCGGCGTCTGTGCGACGGGAGGTTGGGGCCAGGGGACCCTGGTCACCGTCTCG
AGT

FAT 116 Heavy Chain Amino Acid sequence SEQ ID NO. 266

E V Q L V E F G G G L V K P G E S L R L S
C A A S G F T F S D Y Y M S W I R Q A P G
K G L E W V S Y I S S S S S S Y T N Y A D S
V K G R F T I S R D N A K N S L Y L Q M N
S L R A E D T A V Y Y C A R G G T Q C S F
G V C A T G G W G Q G T L V T V S S

FAT 116 Light Chain DNA and Amino Acid sequences

Identical to FAT 31 (SEQ ID NO.'s 87 and 88)

FAT 117 Heavy Chain DNA Sequence SEQ ID NO. 267

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGAGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTGACCACCACATGGACTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTGGCCAACATAAACCGAGATGGAAGTGACTACCGCTATGTG

GACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACGACGCCAAGAACTCACTGTATCTCC
AAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCGGGTTCTG
CCTTAACCCTGTGTGTTATCATGGAGGTTGGGGCCAGGGAACCCTGGTCACCGCCTCCTCA

FAT 117 Heavy Chain Amino Acid sequence SEQ ID NO. 268

Q V Q L V E S G G G V V Q P G G S L R L S
C A A S G F T F S D H H M D W V R Q A P G
K G L E W V A N I N R D G S D Y R Y V D S
V K G R F T I S R D D A K N S L Y L Q M N
S L R A E D T A V Y Y C A R G G F C L N P
V C Y H G G W G Q G T L V T A S S

FAT 117 Light Chain DNA and Amino Acid sequence

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

FAT 118 Heavy Chain DNA Sequence SEQ ID NO. 269

GAGGTGCAGCTGGTGGAGTTTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTT
CCTGCAAGGCCTCTGATTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCCCC
TGGACAAGGGCTTGAGTGGATGGGATGGAGCAGCGCTAACGATGGTAACACAAACTATGCA
CAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGG
AGTTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGGCGGGCTGCC
CTGCCCTTGTGCTGCCTGTTGCTCCGGAGGTTGGGGCCAGGGACCCTGGTCACCGTCTCC
TCA

FAT 118 Heavy Chain Amino Acid sequence SEQ ID NO. 270

E V Q L V E F G A E V K K P G A S V K V S
C K A S D Y T F T S Y G I S W V R Q A P G
Q G L E W M G W S S A N D G N T N Y A Q K

L Q G R V T M T T D T S T S T A Y M E L R
S L R S D D T A V Y Y C A R G G L P C P C
A A C C S G G W G Q G T L V T V S S

FAT 118 Light Chain DNA and Amino Acid sequences

Identical to FAT 30 (SEQ ID NO.'s 83 and 84)

INTERNATIONAL SEARCH REPORT

Ir onal Application No
PCT/GB 00/03900

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C07K16/28 C12N5/10 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, STRAND, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HOOGENBOOM H R ET AL: "Selection - dominant and nonaccessible epitopes on cell-surface receptors revealed by cell-panning with a large phage antibody library." EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 MAR) 260 (3) 774-84. , XP000978815 abstract page 774, column 1, line 1 -page 779, column 1, paragraph 1 page 780, column 1, line 1 -page 783, paragraph 2 --- -/--	1-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2001	30/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muller-Thomalla, K

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PILLION D J ET AL: "Immunofluorescent studies of the rat adipocyte cell surface." INTERNATIONAL JOURNAL OF IMMUNOPHARMACOLOGY, (1984) 6 (3) 193-204. , XP000978782 abstract page 193, column 2, last paragraph -page 194, column 2, paragraph 1 ---	1-37
Y	US 5 631 009 A (CRYER ANTHONY ET AL) 20 May 1997 (1997-05-20) column 9, line 4 -column 12, line 30; figure 3 ---	1-37
Y	US 5 102 658 A (FLINT DAVID J) 7 April 1992 (1992-04-07) column 2, line 47 -column 4, line 53 ---	1-37
Y	LOGTENBERG T ET AL: "Detecting novel cell surface antigens using phage antibody display" IMMUNOTECHNOLOGY,NL,ELSEVIER SCIENCE PUBLISHERS BV, vol. 2, no. 4, 1 November 1996 (1996-11-01), page 302 XP004063249 ISSN: 1380-2933 abstract ---	1-37
Y	HOOGENBOOM H R ET AL: "Antibody phage display technology and its applications" IMMUNOTECHNOLOGY,NL,ELSEVIER SCIENCE PUBLISHERS BV, vol. 4, no. 1, 1 June 1998 (1998-06-01), pages 1-20, XP004127382 ISSN: 1380-2933 abstract page 2, column 1, last paragraph -page 13, column 2, paragraph 1 ---	1-37
Y	HOOGENBOOM H R: "Designing and optimizing library selection strategies for generating high-affinity antibodies" TRENDS IN BIOTECHNOLOGY,GB,ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 15, no. 2, 1 February 1997 (1997-02-01), pages 62-70, XP004034115 ISSN: 0167-7799 the whole document --- -/--	1-37

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
T	EDWARDS B M ET AL: "Isolation and tissue profiles of a large panel of phage antibodies binding to the human adipocyte cell surface" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL, vol. 245, no. 1-2, 1 November 2000 (2000-11-01), pages 67-78, XP004218809 ISSN: 0022-1759	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: Part of claims 1-17 and 20-37

The scope of part of present claims 1-17 and 20-37 relate to an extremely large number of possible compounds and methods. In fact, claim 1 and all claims related thereto contain so many options amongst the cited claimed amino acid sequences and possible combinations thereof, that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search for the first invention has been carried out for those parts of the application which could be considered to be clear and concise, namely a panel of 10 "binding members" which each contain one of the VH-CDR sequences shown in Table 4, but restricted to those VH-CDRs of the "FATs" indicated in claim 18, as well as the corresponding ten single binding members (claim 26 onwards) with the distinct VH variable domains as indicated in claim 19 (bringing the present search up to 20 distinct sequence searches in the relevant databases, namely 10 CDR3 sequences (see claim 18) and in addition the corresponding antibody VH variable domains (see claim 19)). In this context it should be noted that the present application as filed does not appear to highlight the relevance of any further combination of 10 binding partners which might be considered essential/relevant to carry out the invention. In this respect, it should also be noted that the relevance of the chosen combination as claimed in claims 18 and 19 is per se not ad hoc apparent from the description and examples of the application as filed, but was chosen as they appeared as the only specific combination of least ten binding members in the present claimed subject-matter.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/03900

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5631009 A	20-05-1997	AT 175422 T	15-01-1999
		AU 664147 B	02-11-1995
		AU 2568692 A	27-04-1993
		CA 2116033 A	01-04-1993
		DE 69228121 D	18-02-1999
		DE 69228121 T	10-06-1999
		DK 597043 T	30-08-1999
		EP 0597043 A	18-05-1994
		WO 9306131 A	01-04-1993
		GB 2259706 A,B	24-03-1993
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